Figure 1

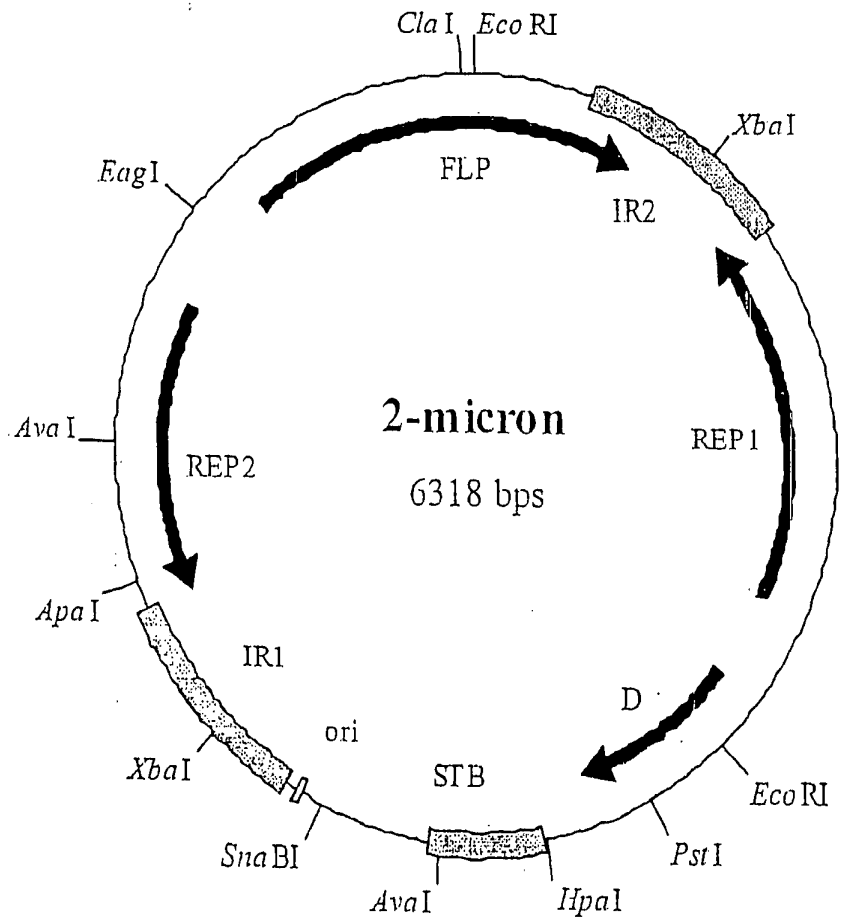


Figure 2

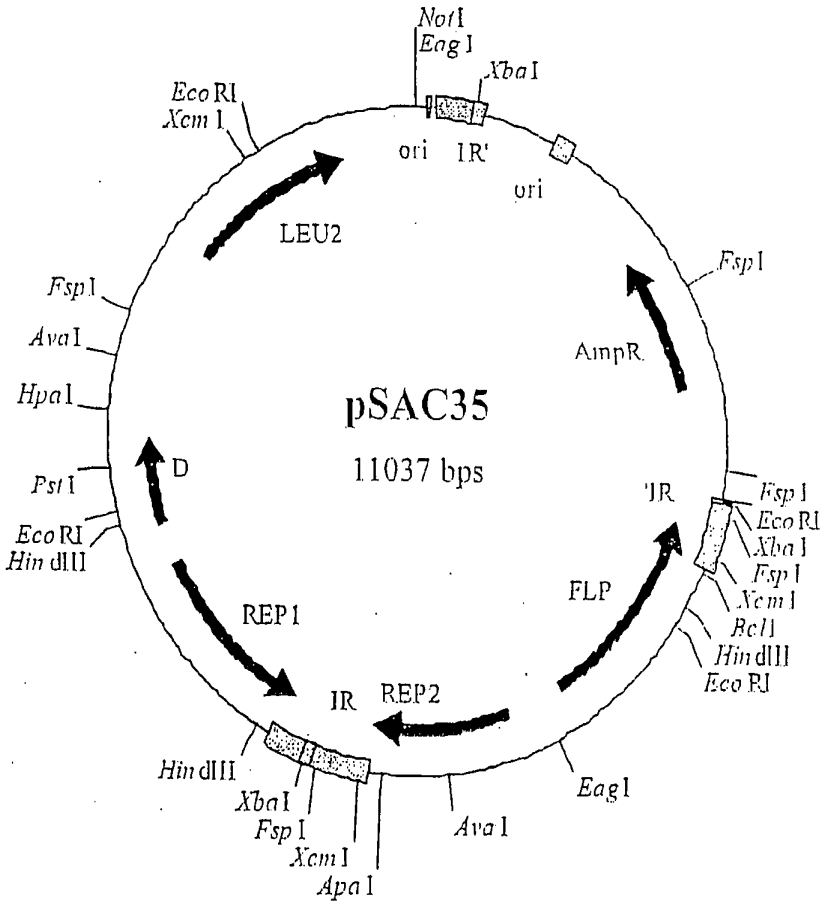
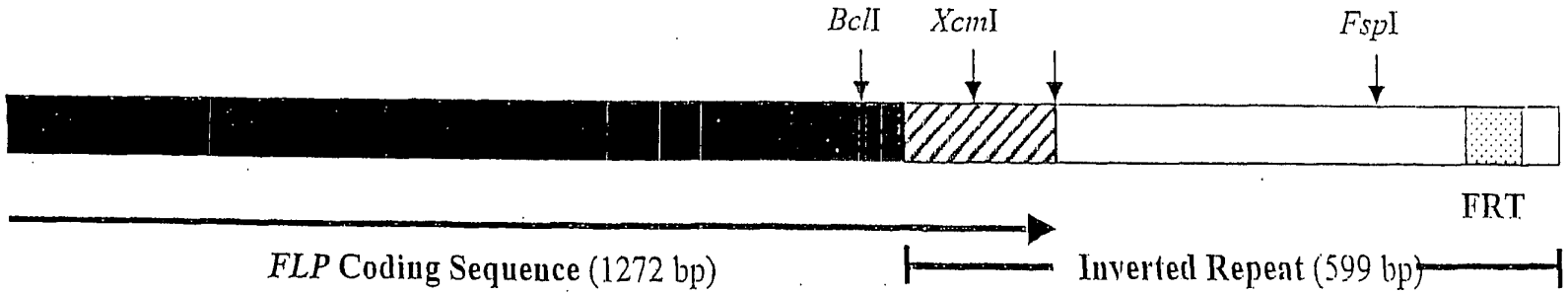


Figure 3

A) Restriction Endonuclease Sites used for DNA Insertions in *FLP* and the *FLP* Inverted



B) Flp Protein (423 amino acid residues)

C) Truncated FLP Protein Products

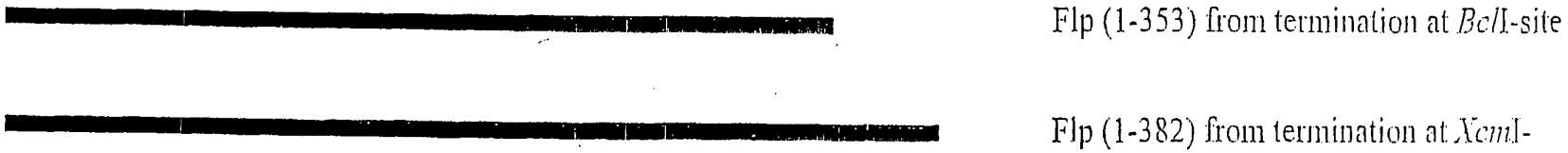


Figure 4

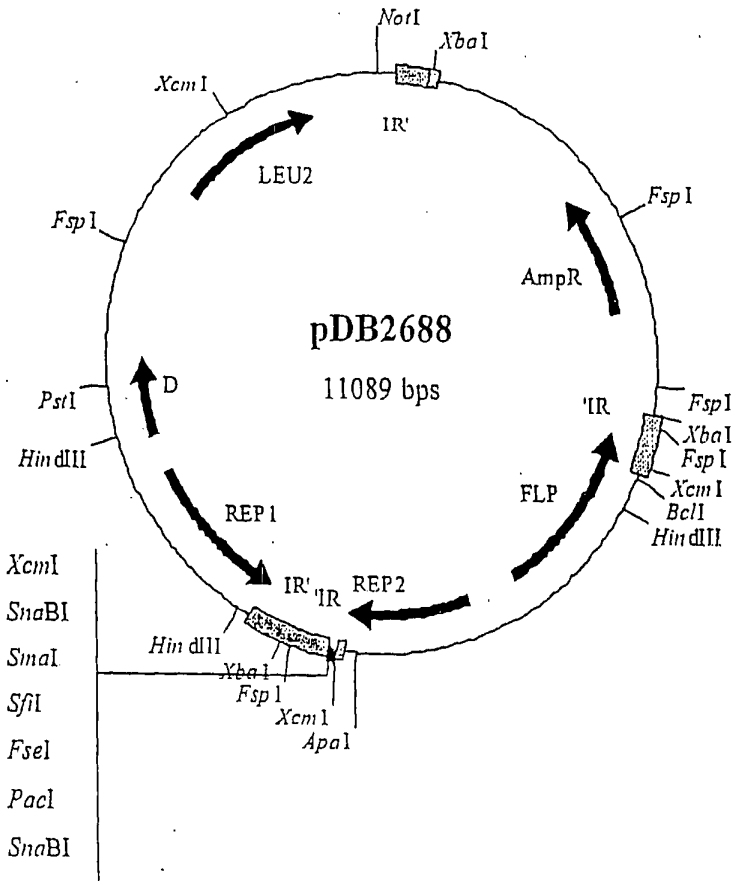


Figure 5

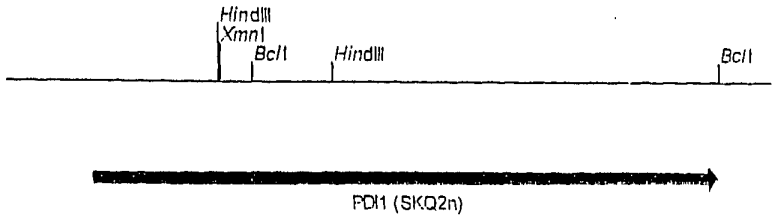


Figure 6

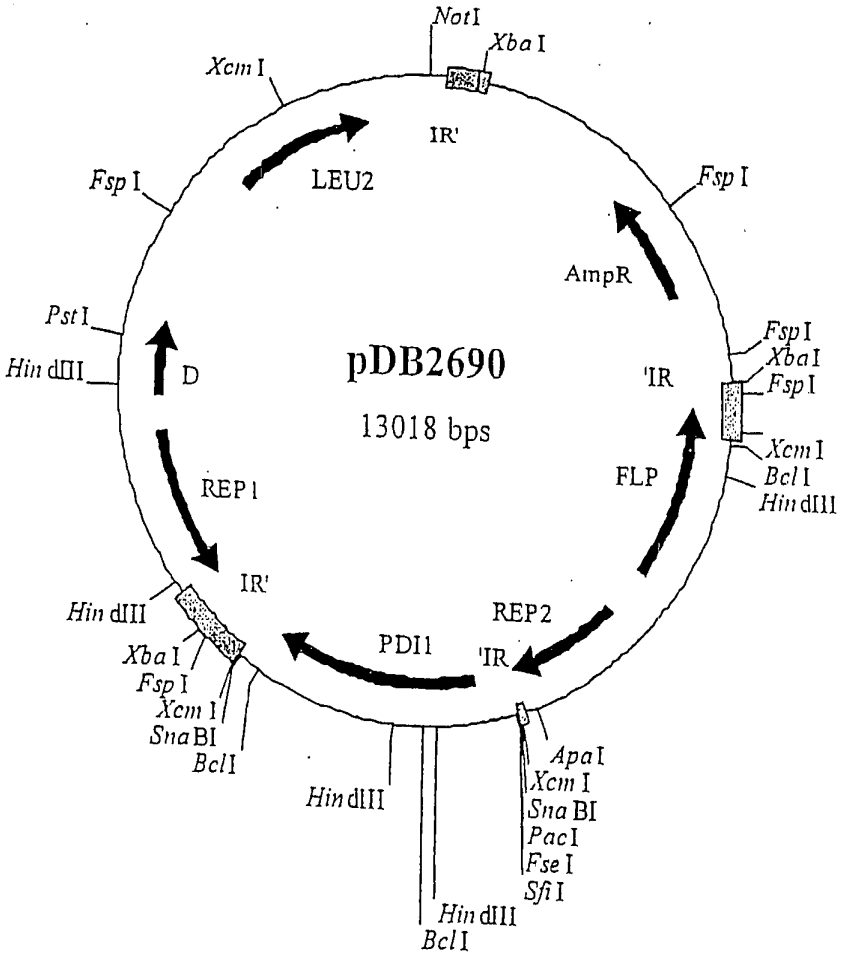


Figure 7

mFL = modified HSA(pre)/MFα1(pro) fusion leader sequence

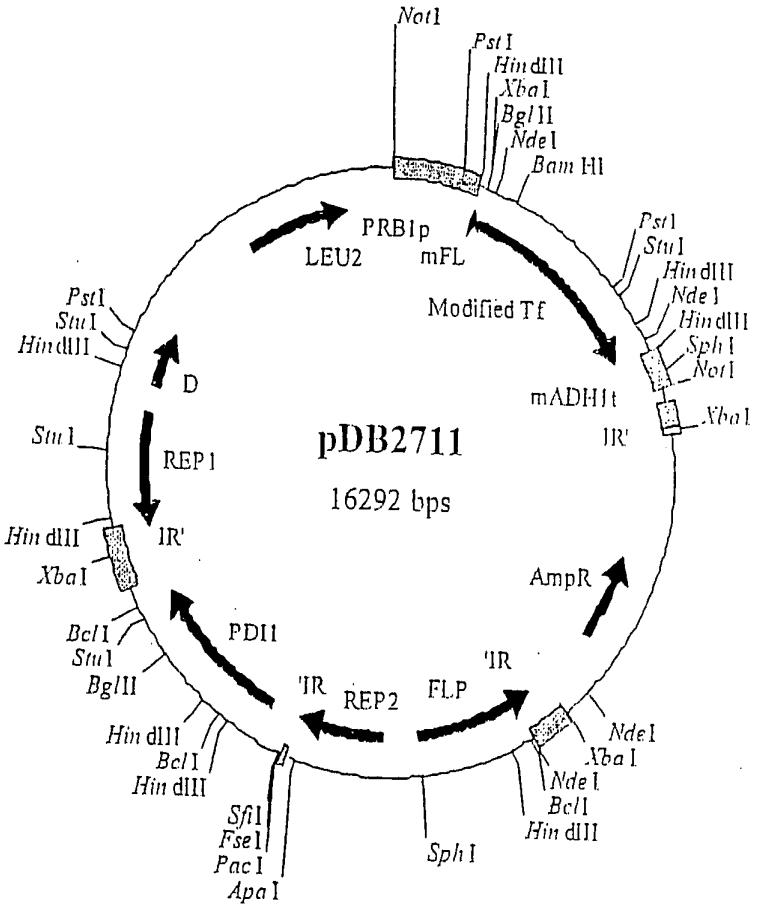


Figure 8

mFL = modified HSA(pre)/MFα1(pro) fusion leader sequence

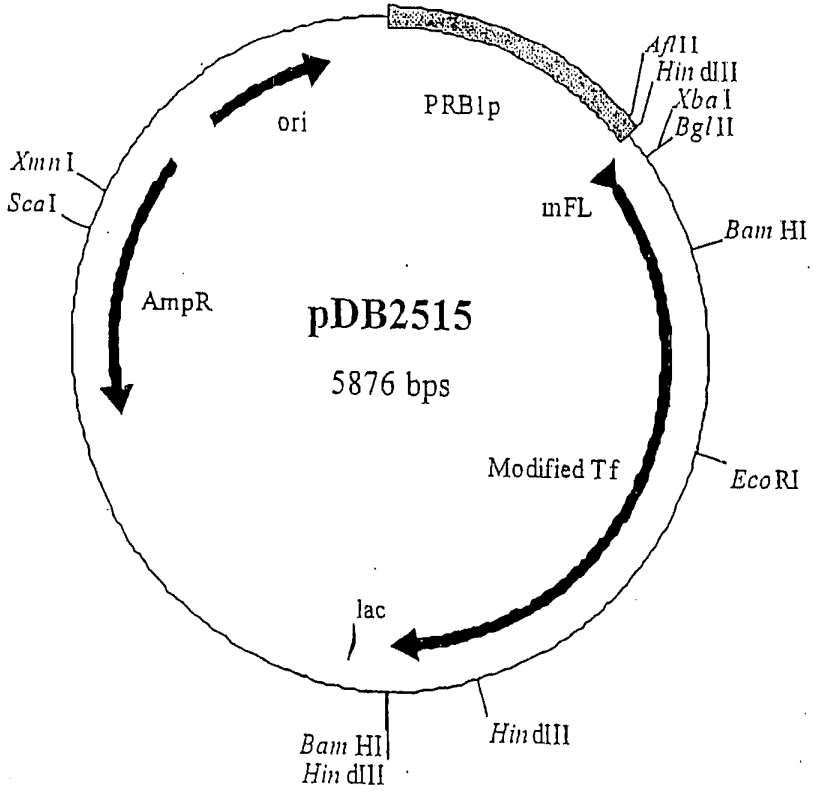


Figure 9

mHSA-pre = modified HSA-pre leader sequence

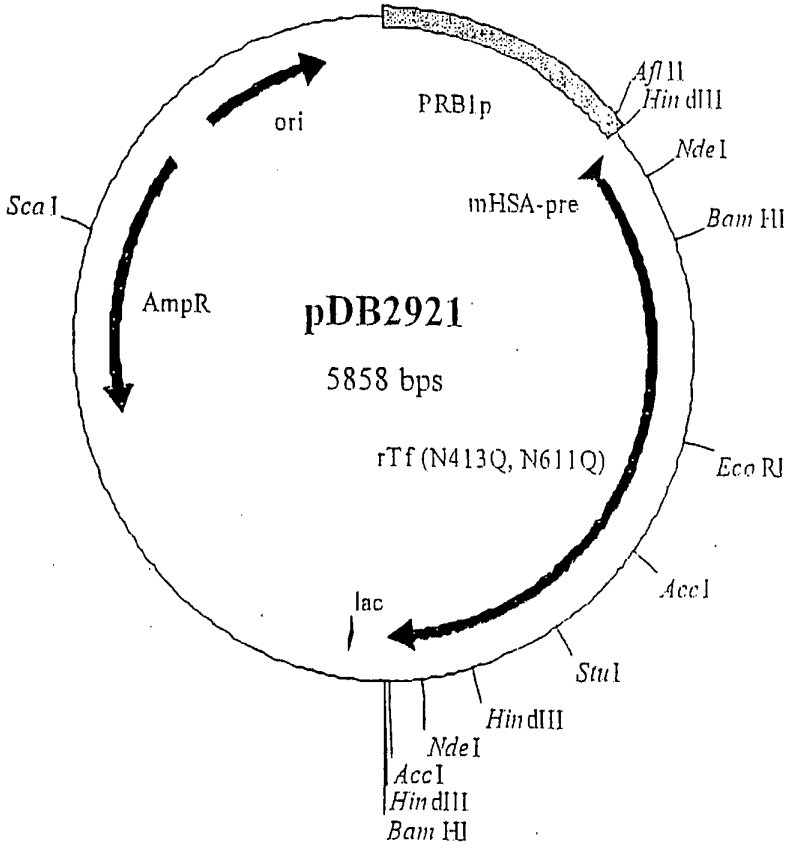


Figure 11

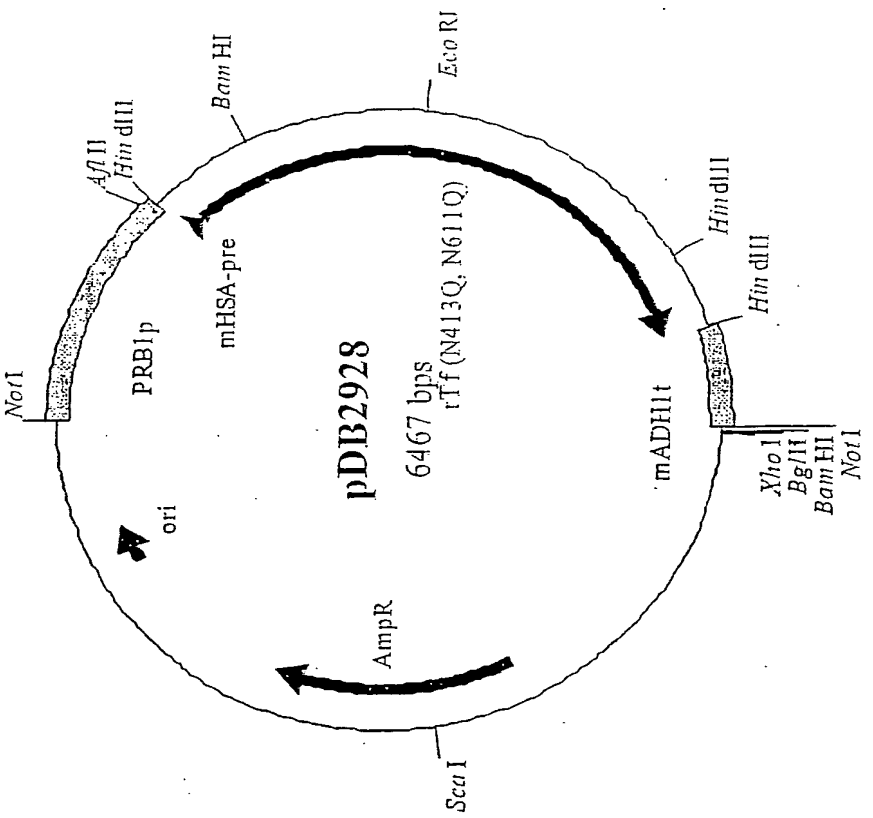


Figure 10

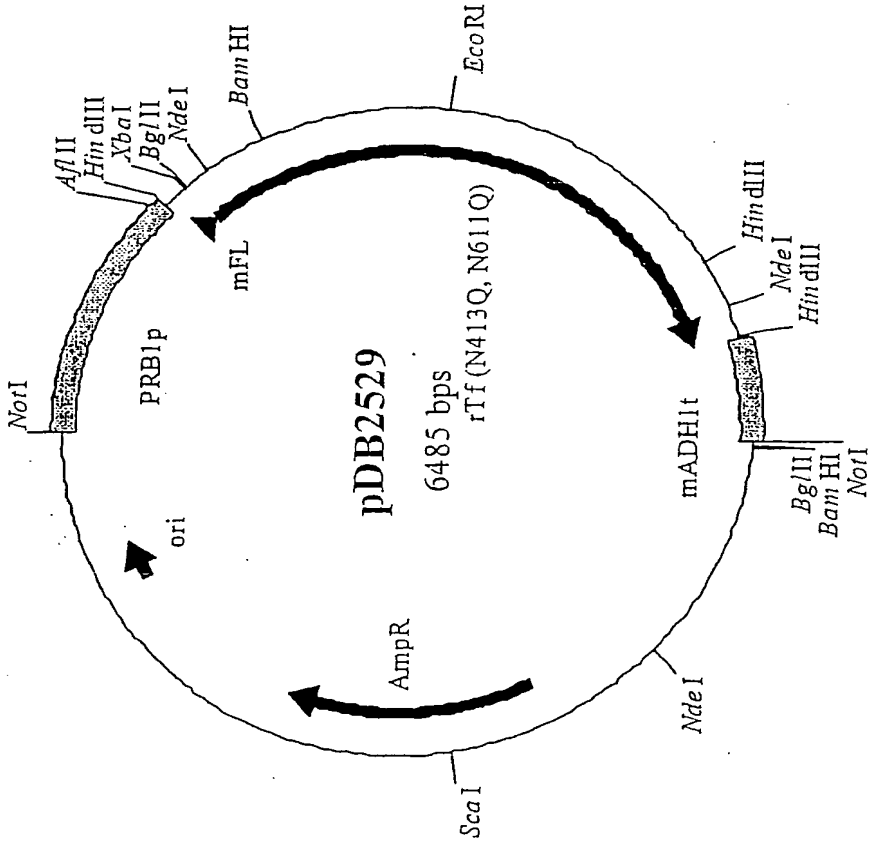


Figure 12

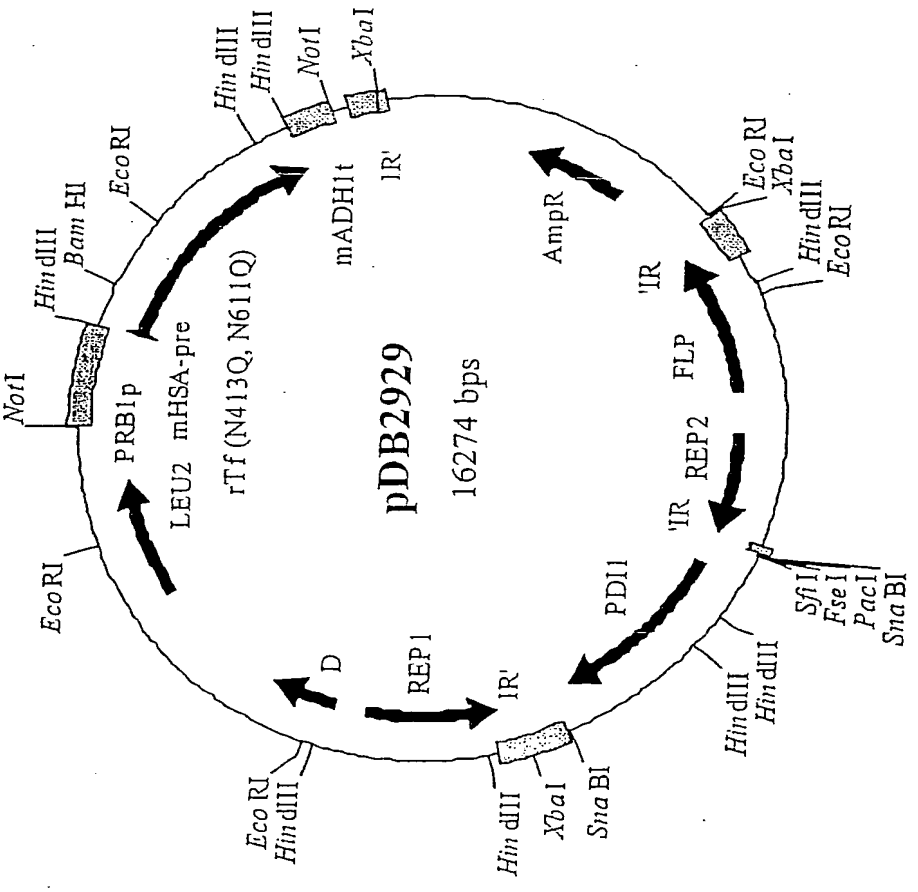
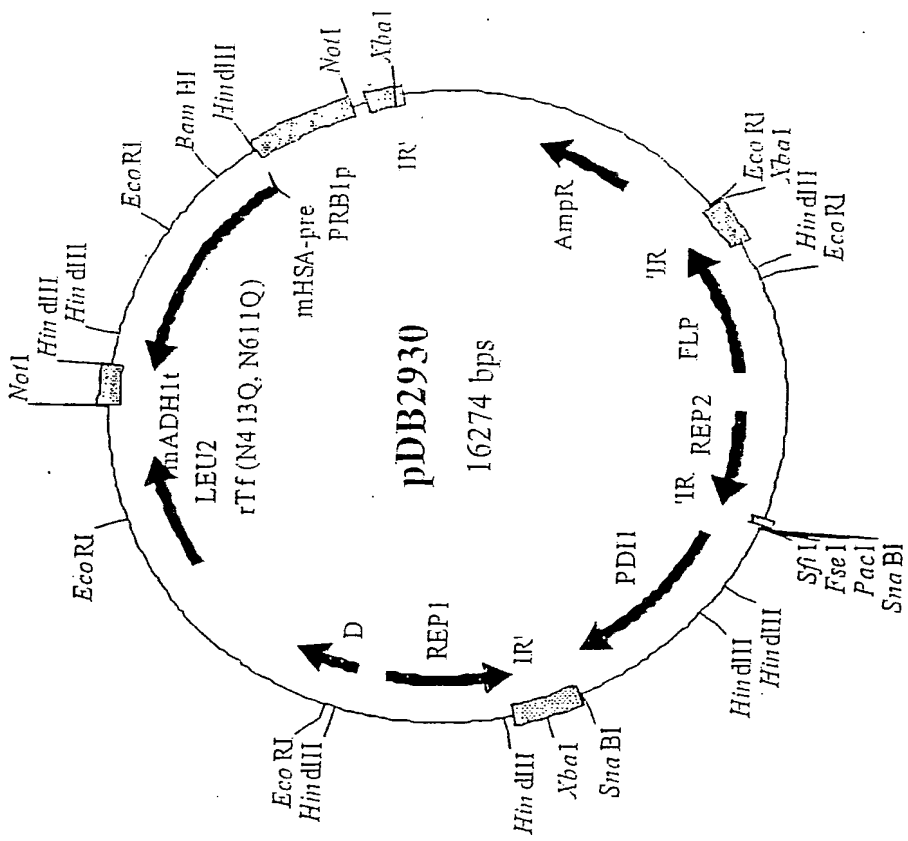


Figure 13



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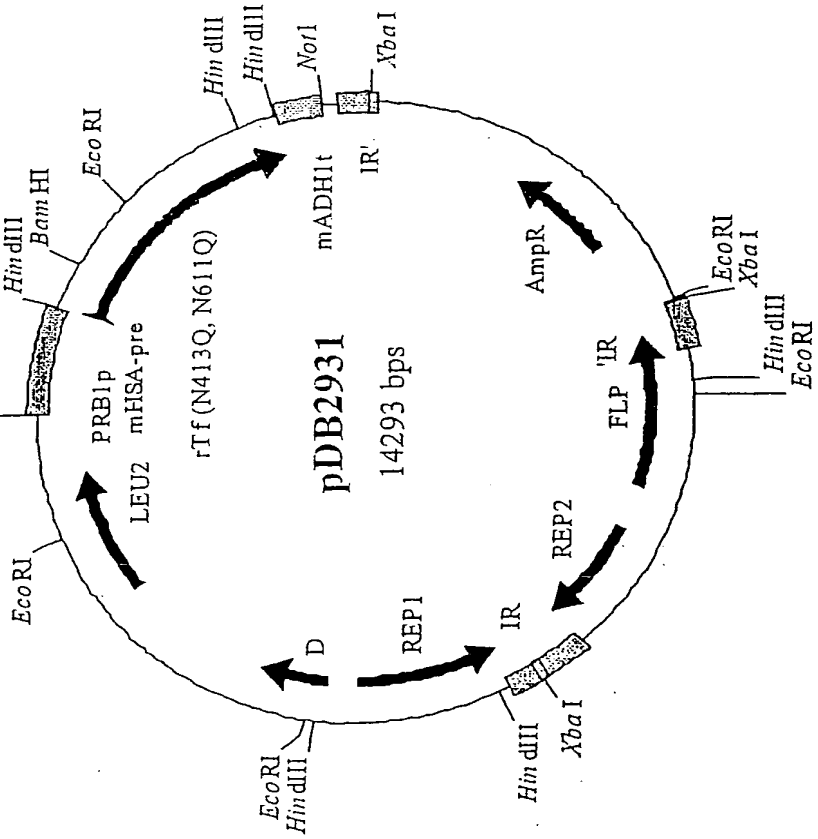
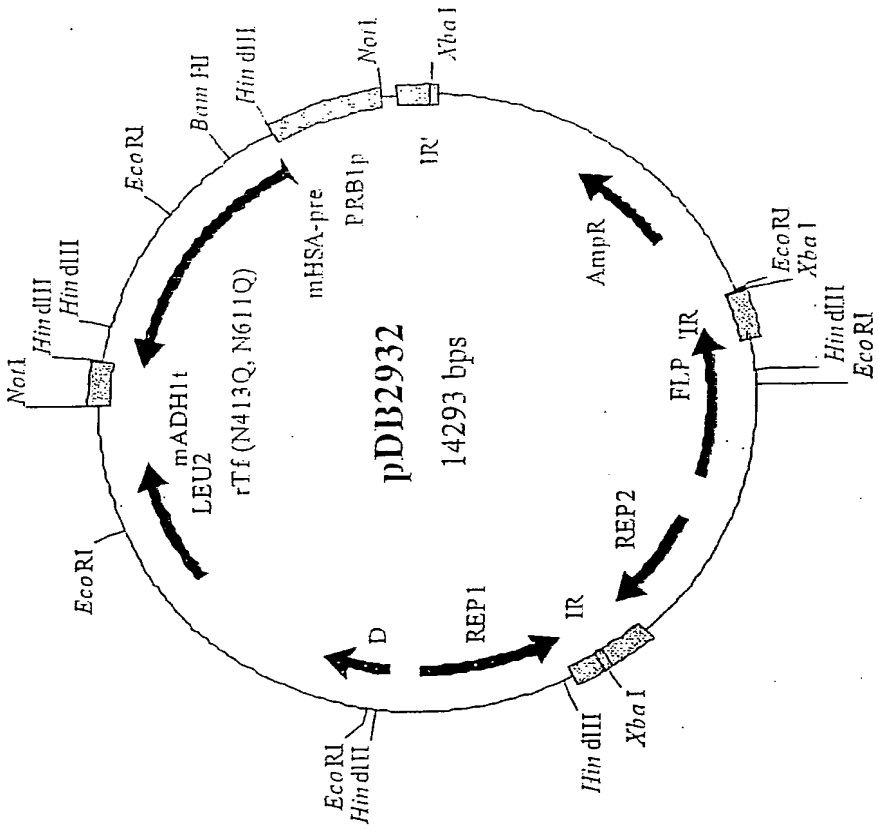


Figure 15



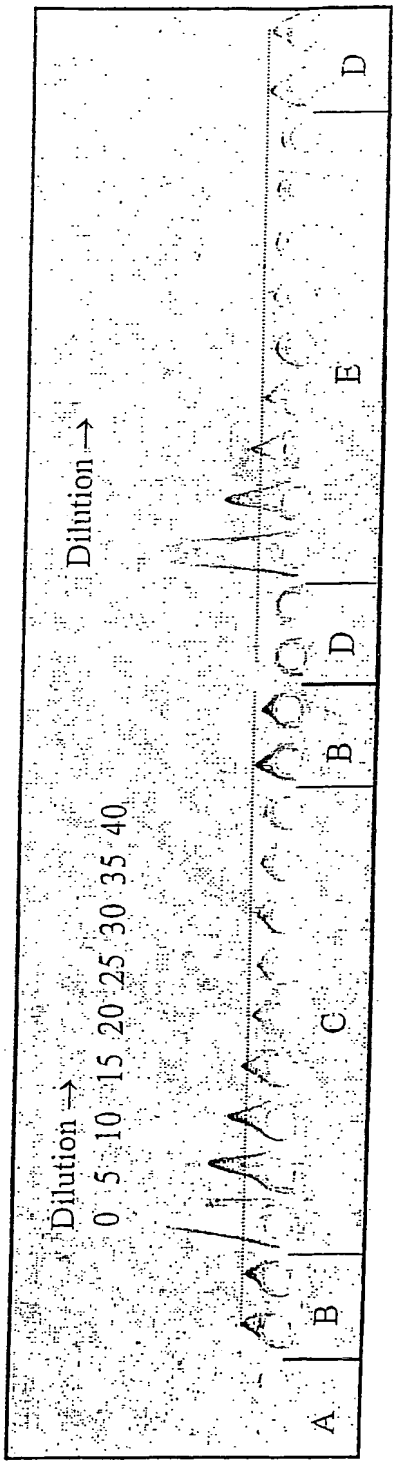


Figure 16

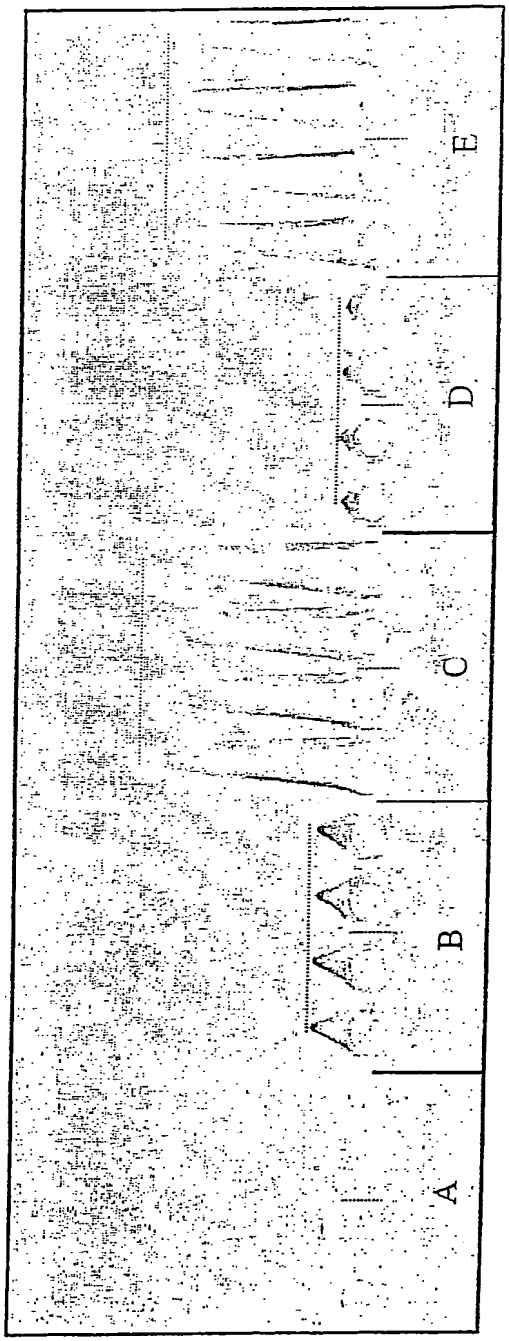


Figure 17

Figure 18

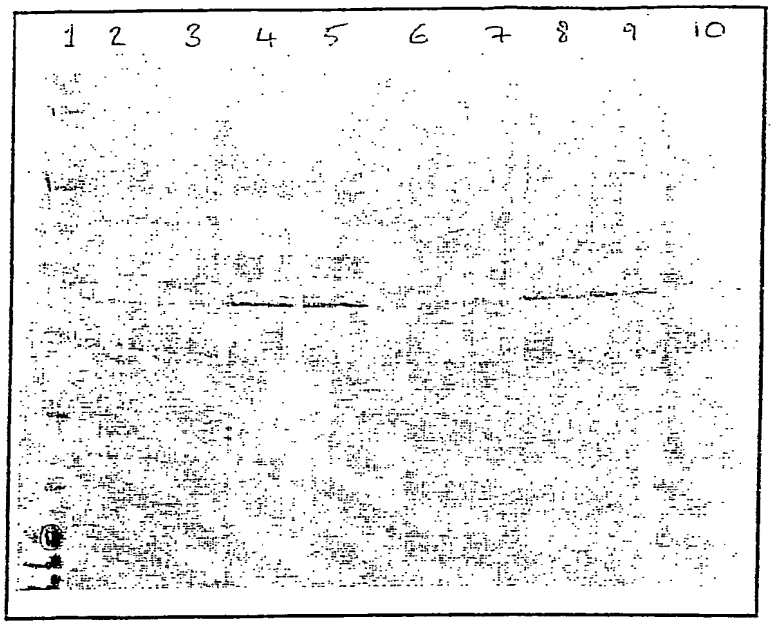
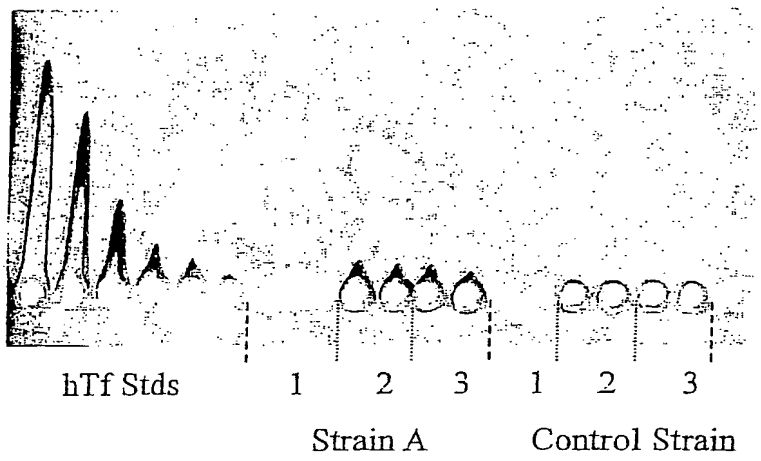
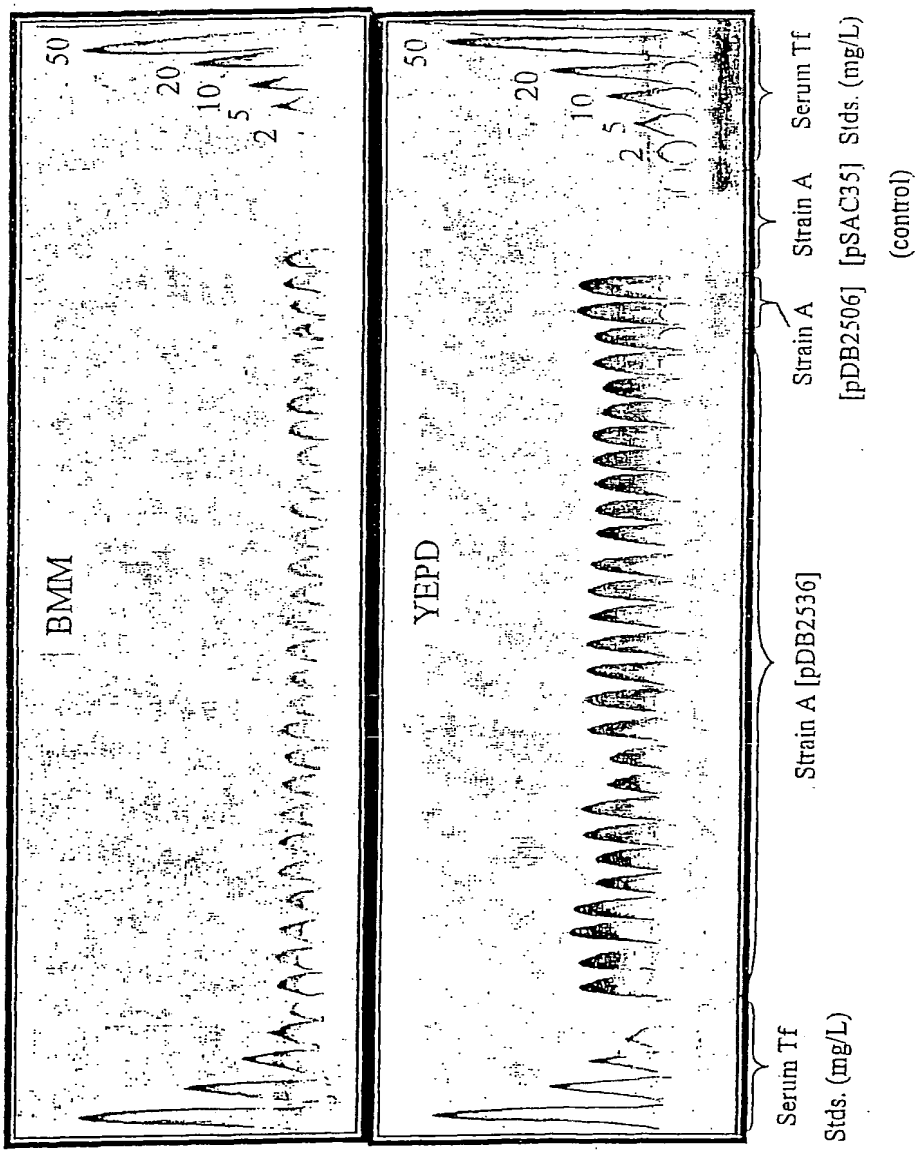


Figure 19

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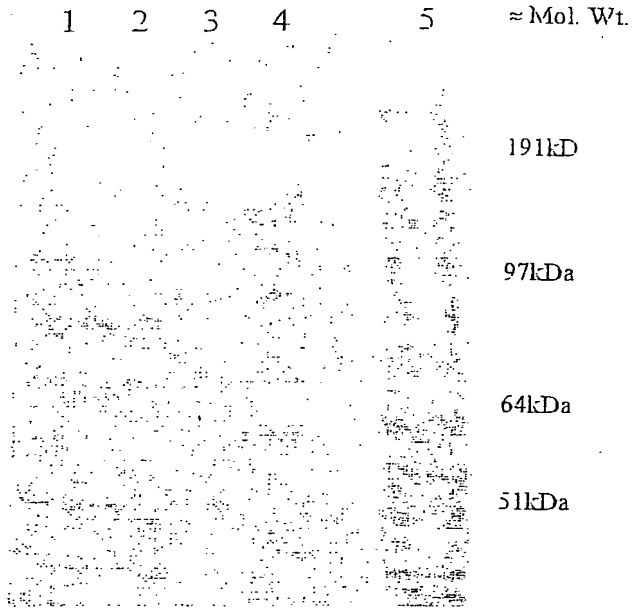
YEPD
50
20
10
5
2
1
Serum Tf
Stds. (mg/L)
Strain A [pDB2536]
Strain A
[pDB2506]
Strain A
[pSAC35]
(control)
Serum Tf
Stds. (mg/L)

Figure 20

Figure 21



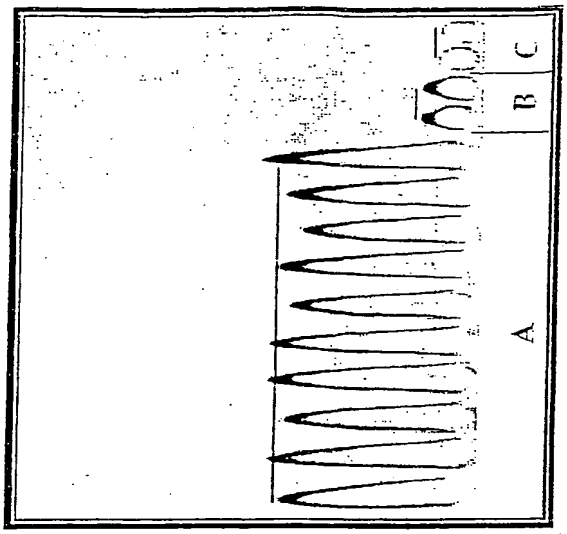


Figure 23

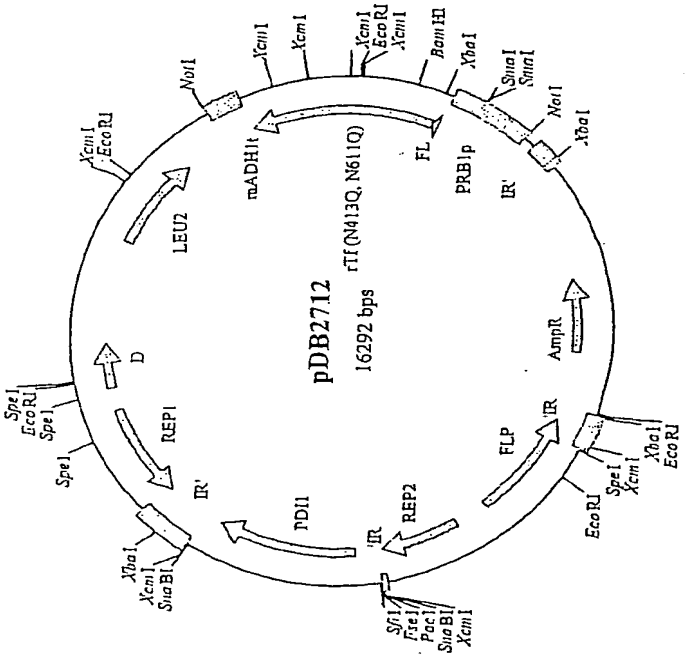


Figure 22

Figure 25

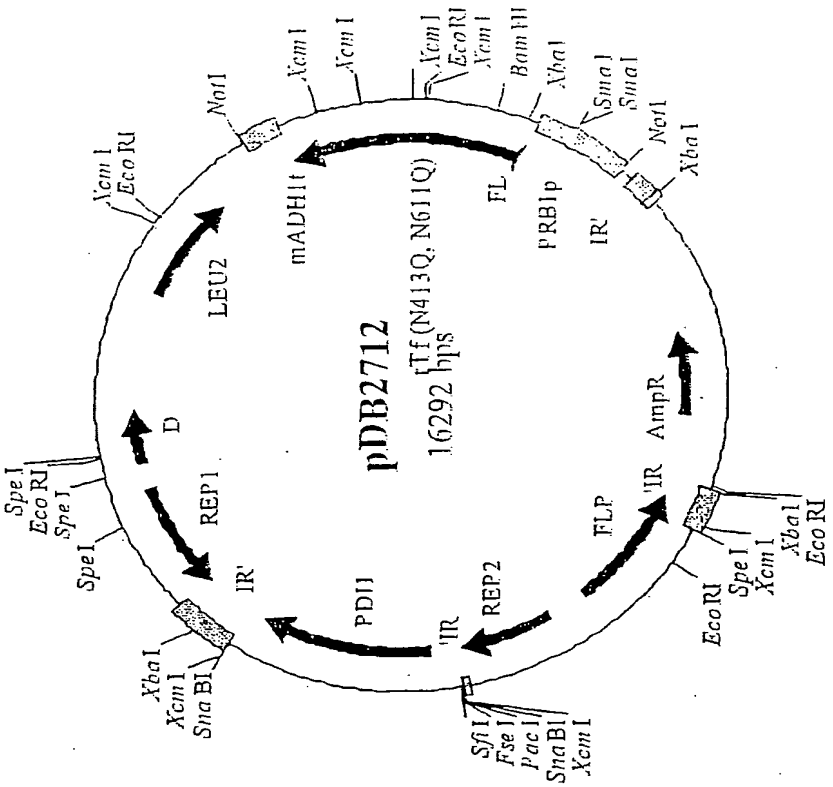


Figure 24

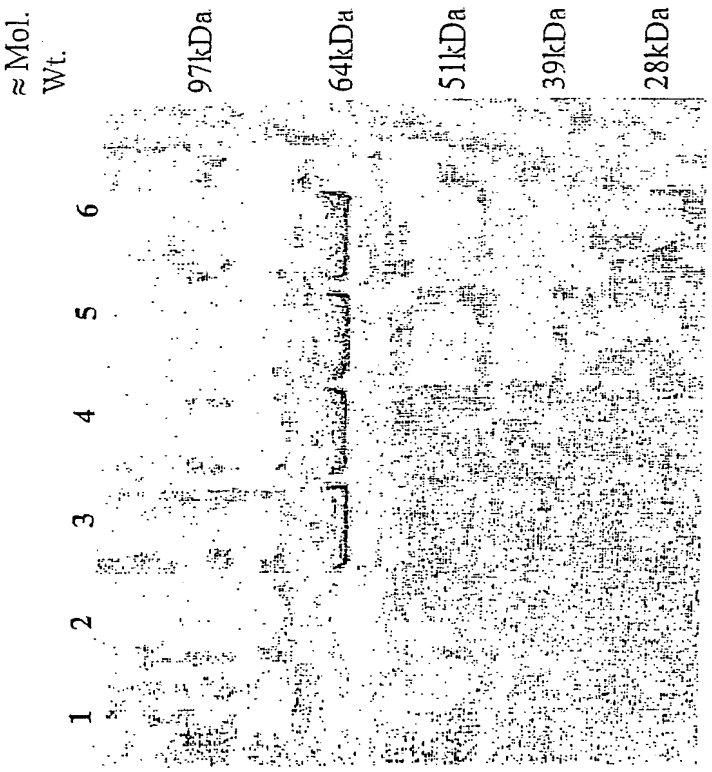
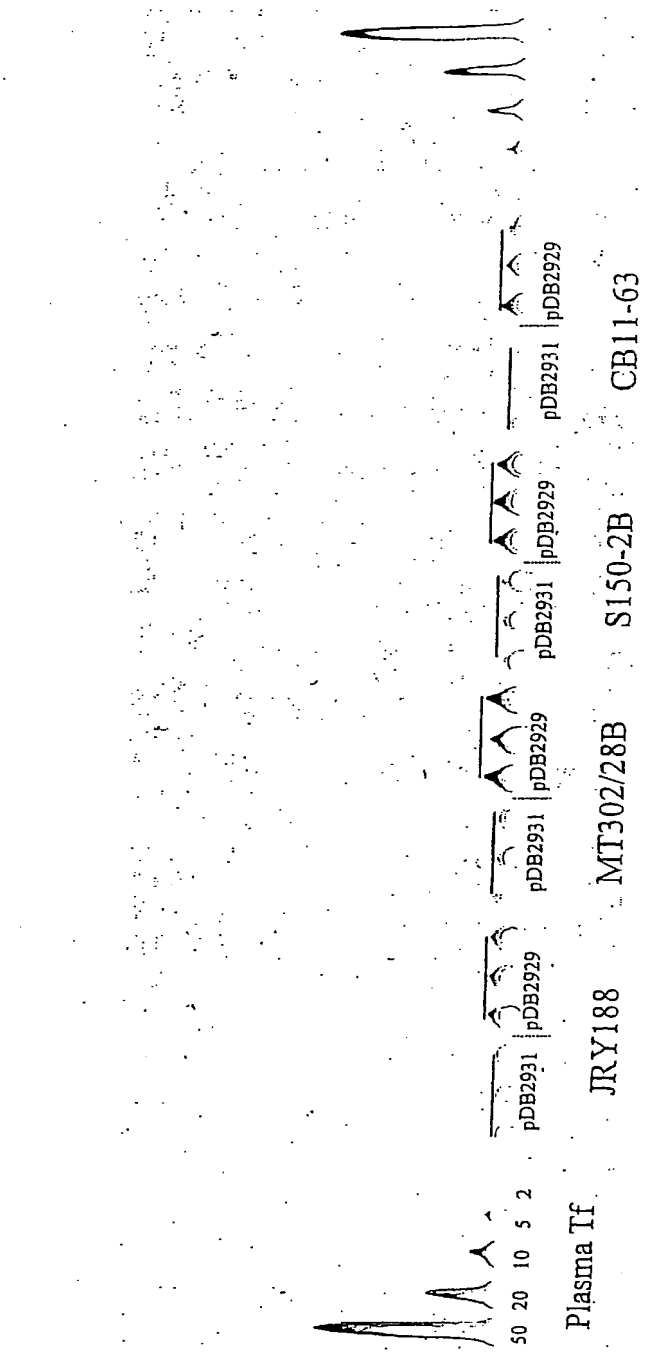


Figure 26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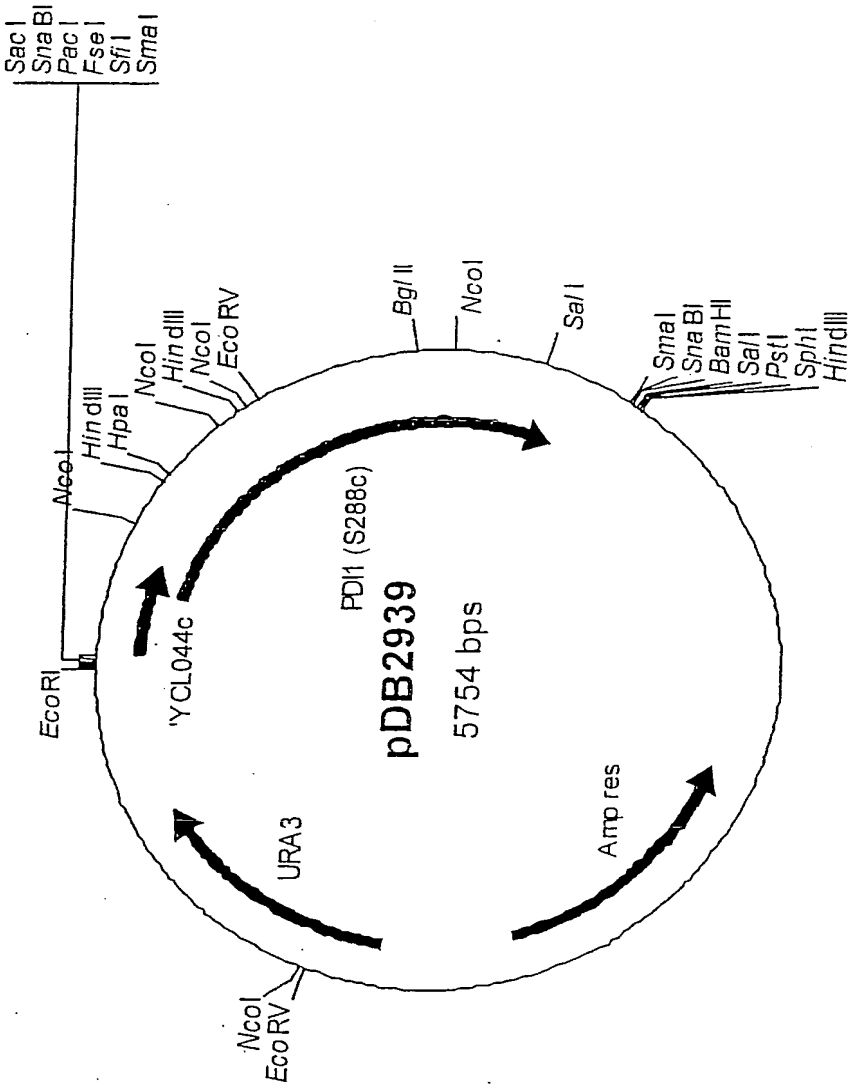
Plasma Tf
JRY188
MT302/28B
S150-2B
CB11-63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Sna BI
Pac I
Fse I
Sfi I
Sma I
Nco I
Hin dIII
Hpa I
Nco I
Hin dIII
Nco I
Eco RV
Bgl II
Nco I
Sal I
Sma I
Sna BI
BamHI
Sal I
Pst I
Sph I
Hind III
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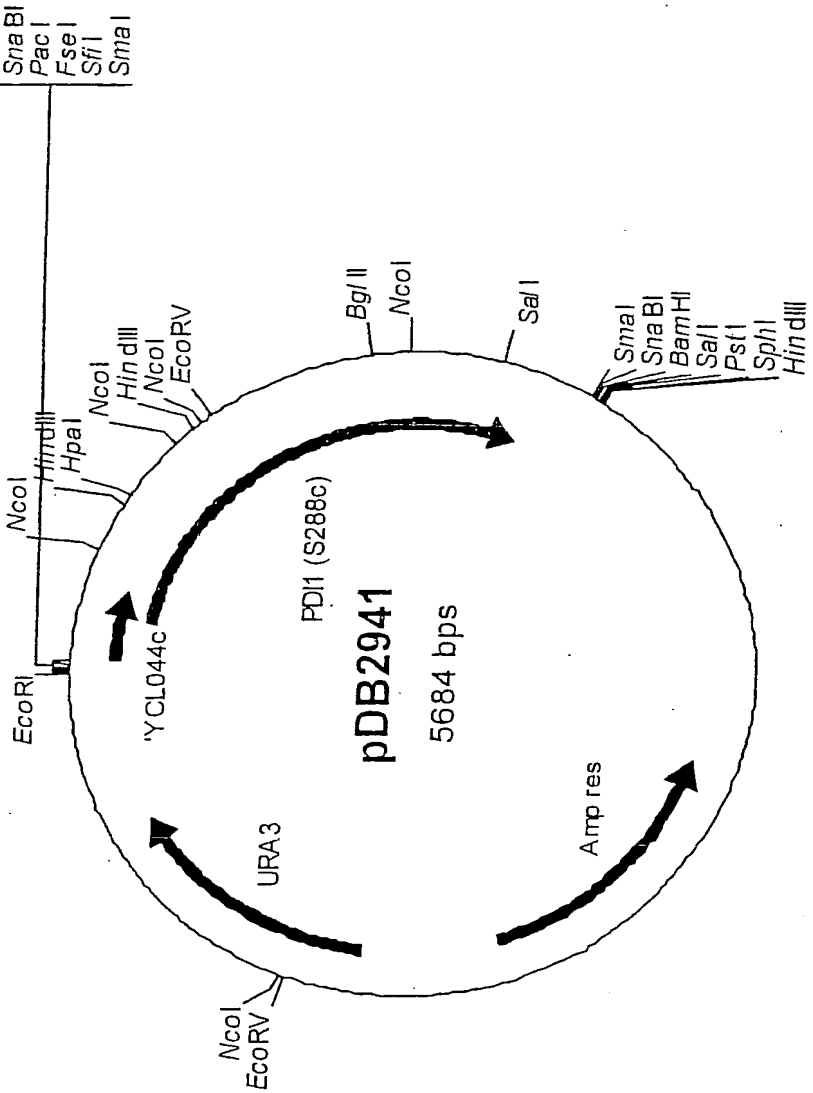
URA3
Amp res
Nco I
Eco RV

Figure 27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BglII
NcoI
SalI
SmaI
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PacI
FseI
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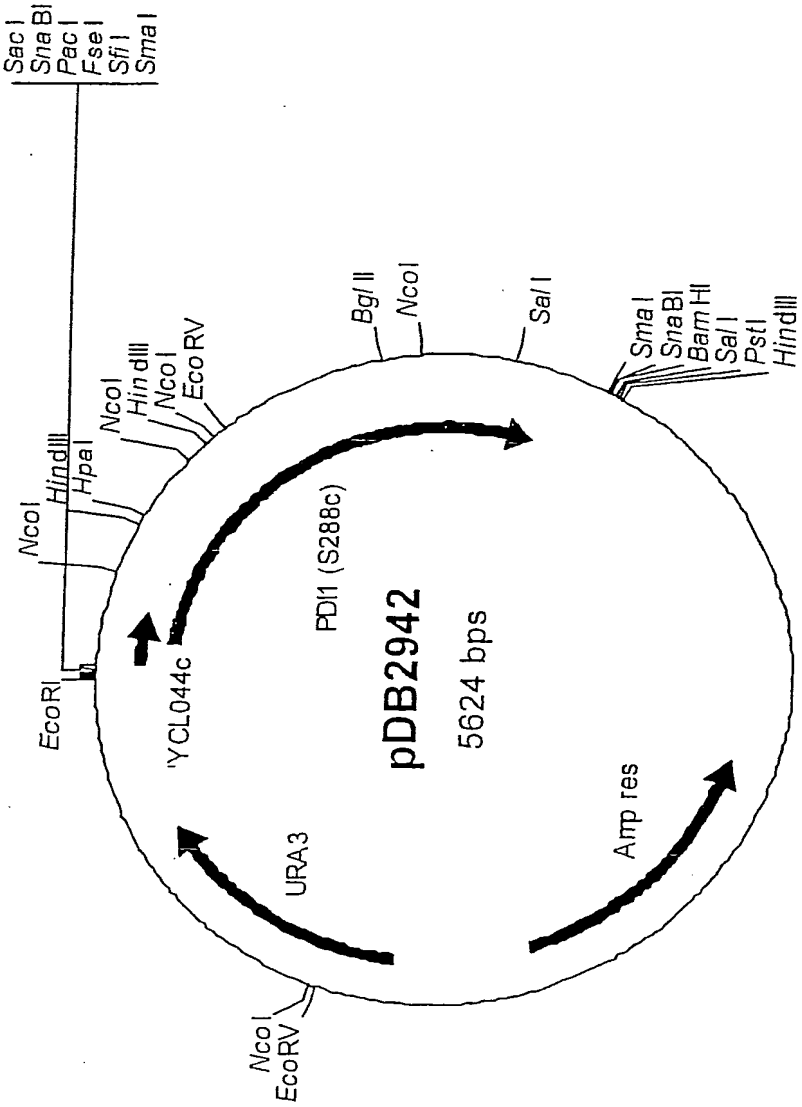
SmaI

Figure 28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Hind III
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Hin dIII
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Eco RV
Bgl II
Nco I
Sal I
Sma I
Sna BI
Bam HI
Sal I
Pst I
Hind III
Eco RI
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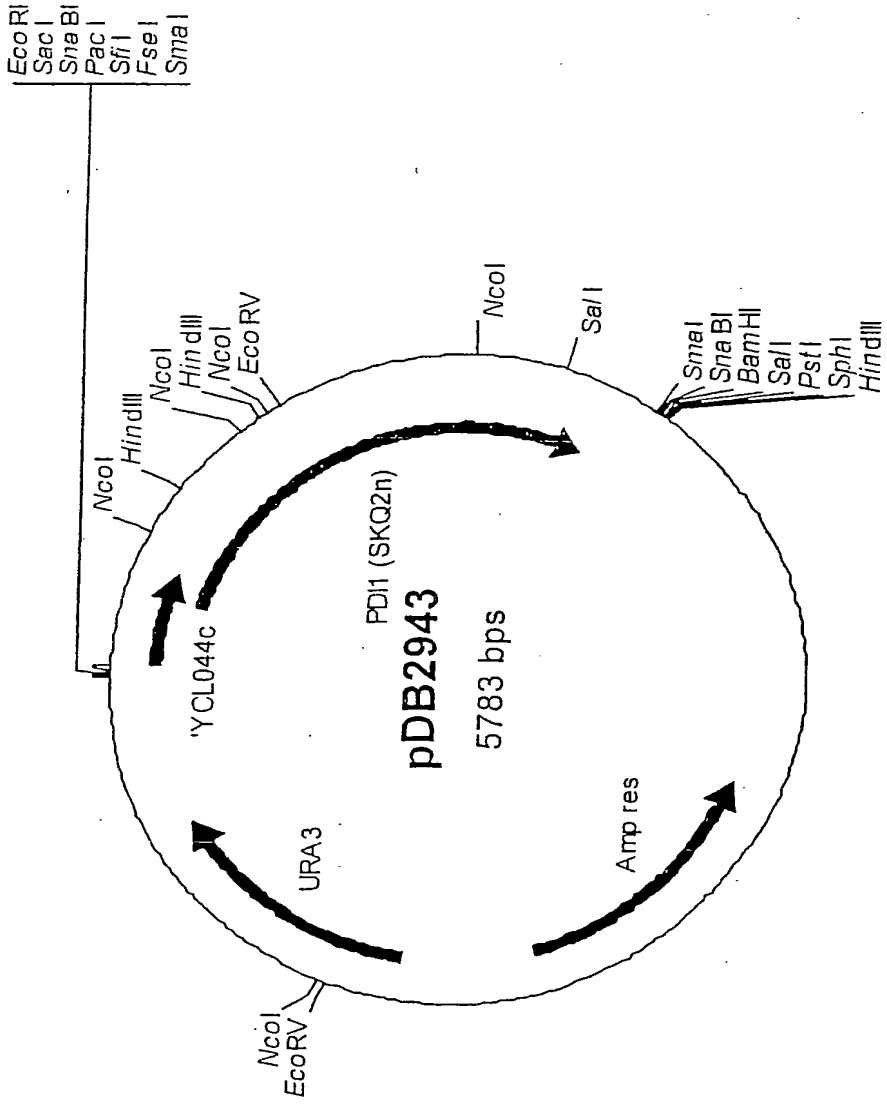
URA3
Amp res
Nco I
Eco RV

Figure 29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SacI
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PacI
SfiI
FseI
SmaI
NcoI
HindIII
NcoI
HindIII
NcoI
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SalI
PstI
SphI
HindIII
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PDI1 (SKQ2n)
pDB2943
5783 bps
URA3
Amp res

Figure 30

Figure 31

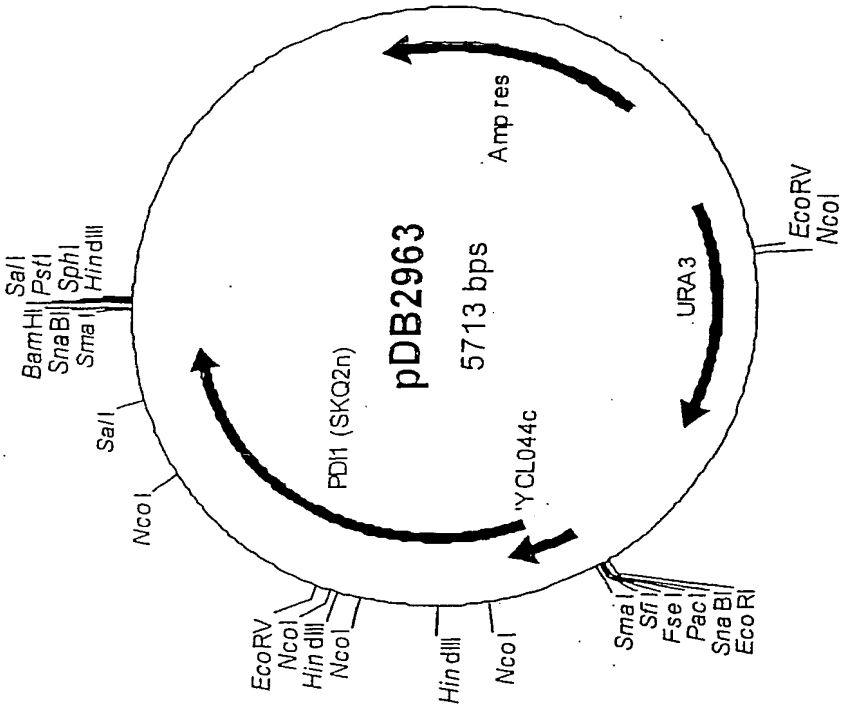
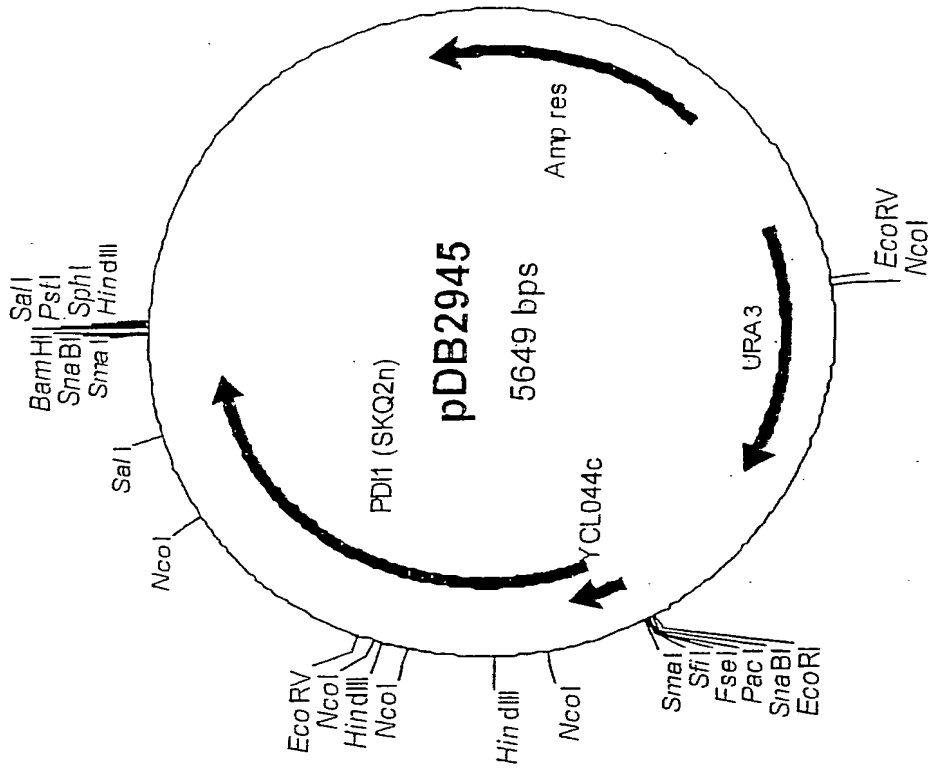


Figure 32



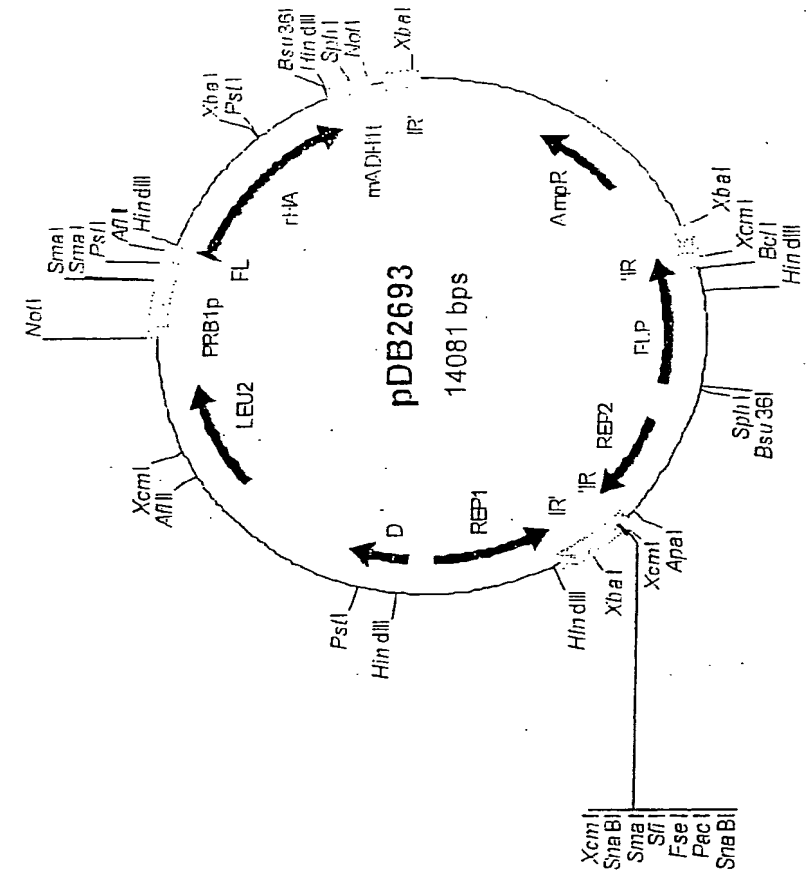


Figure 34

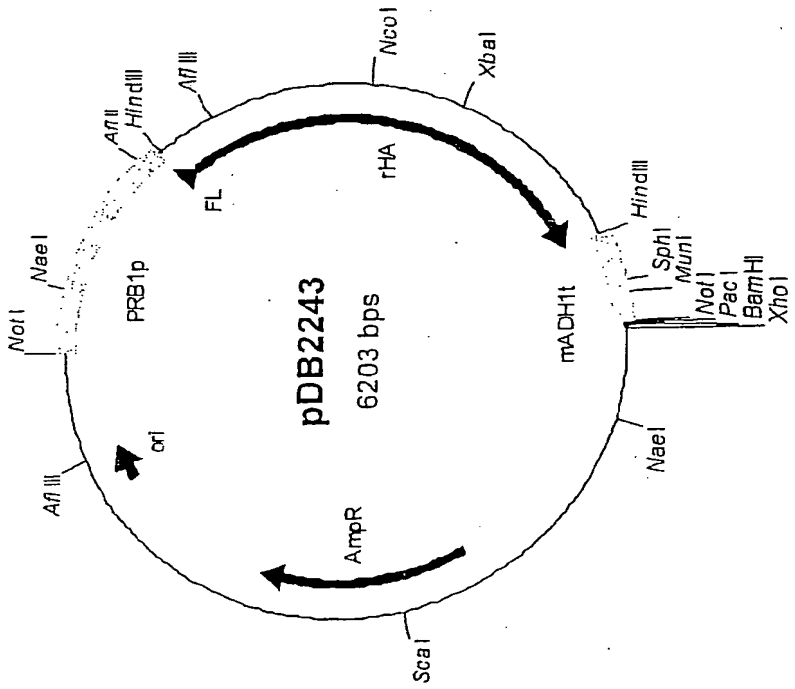


Figure 33

FIGURE 35

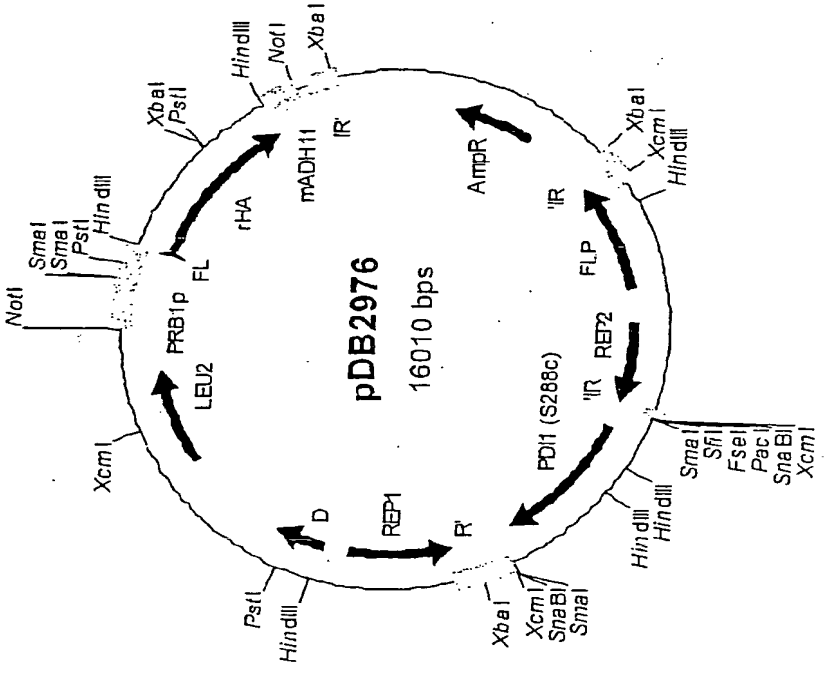


FIGURE 36

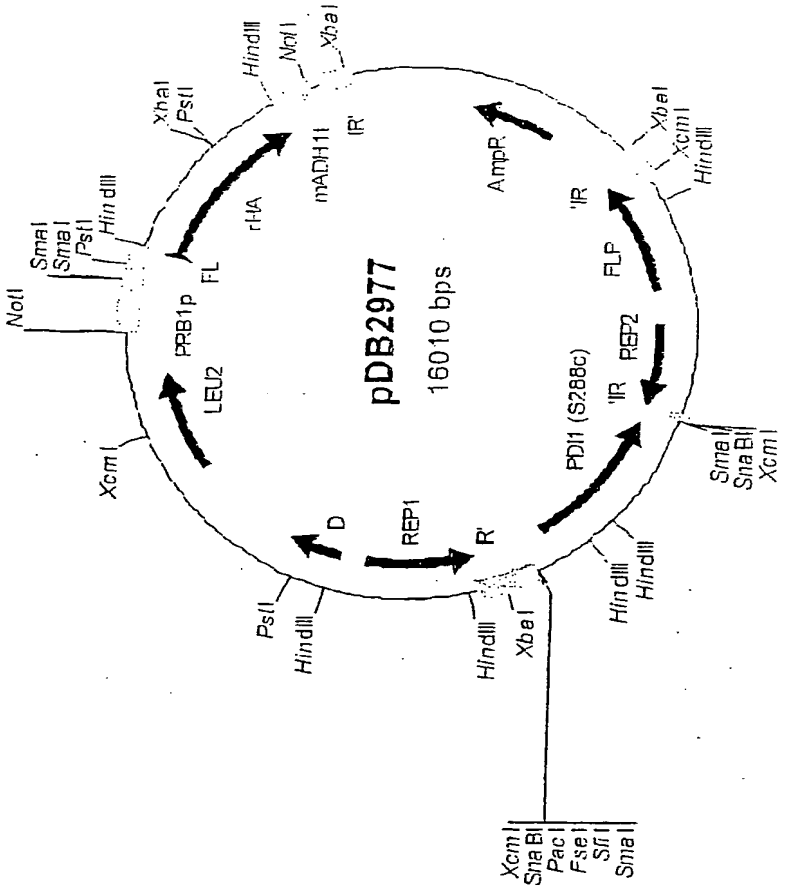


FIGURE 37

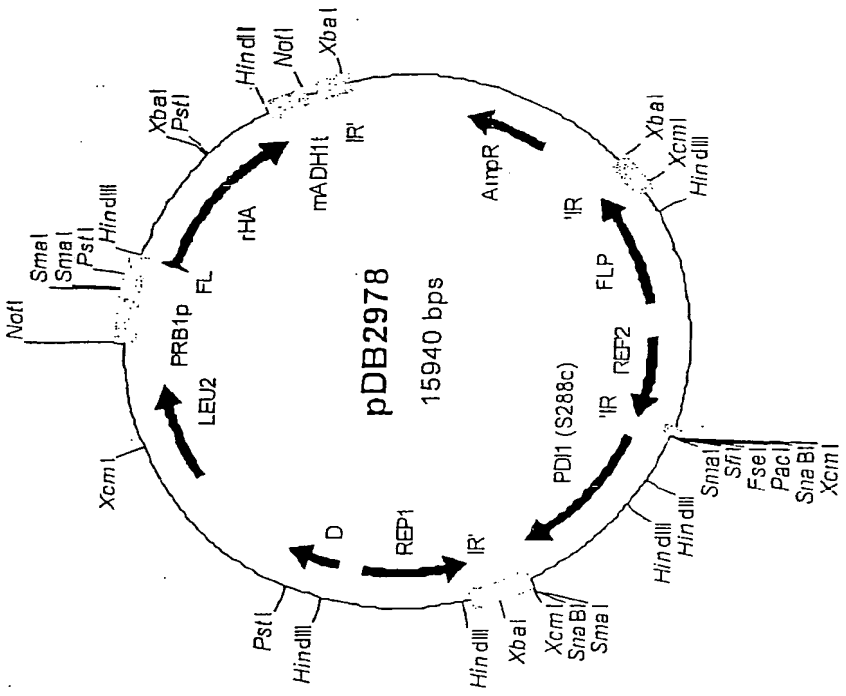


FIGURE 38

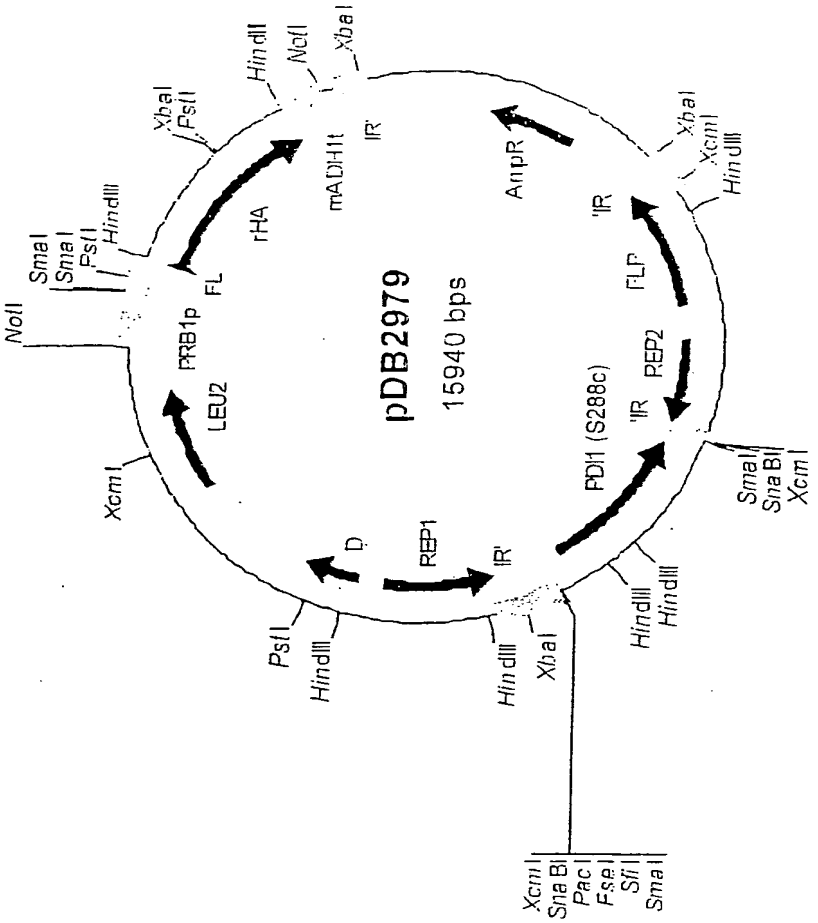


FIGURE 40

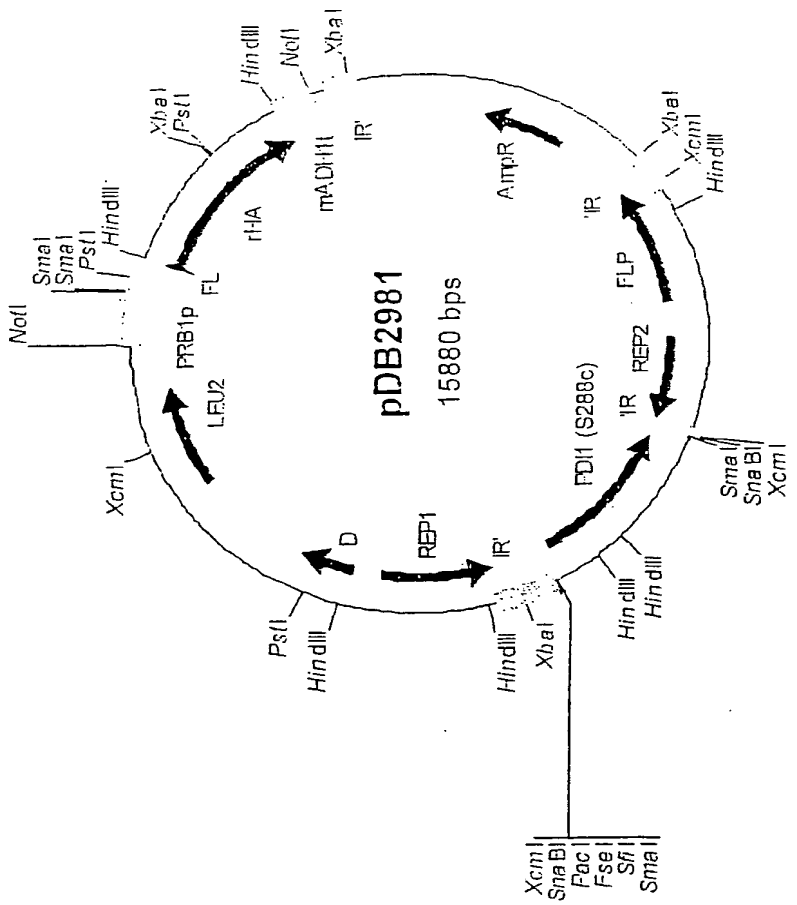


FIGURE 39

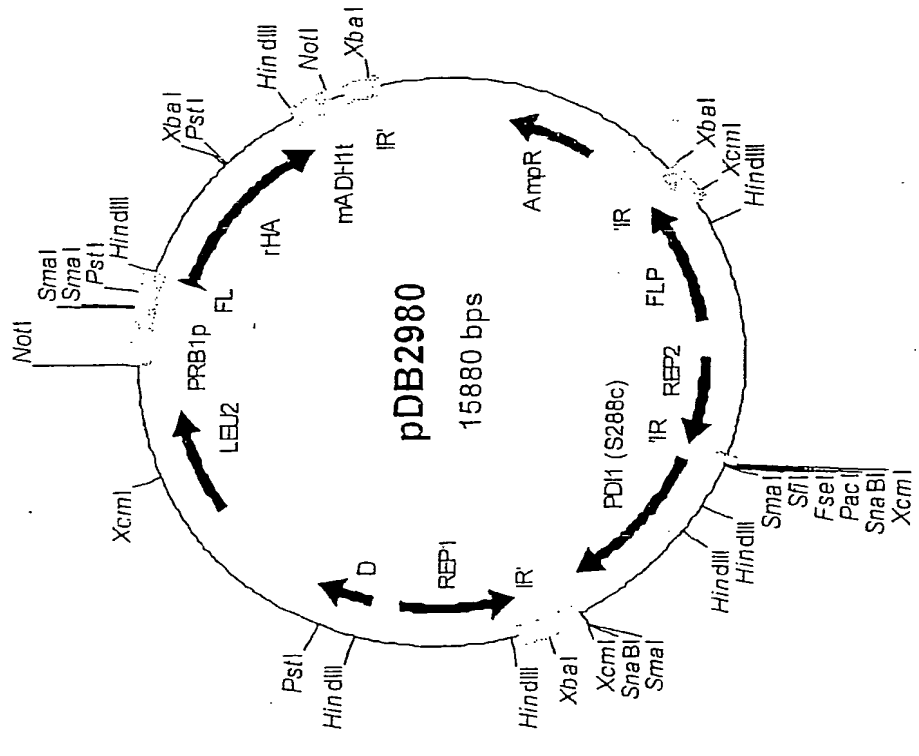


FIGURE 41

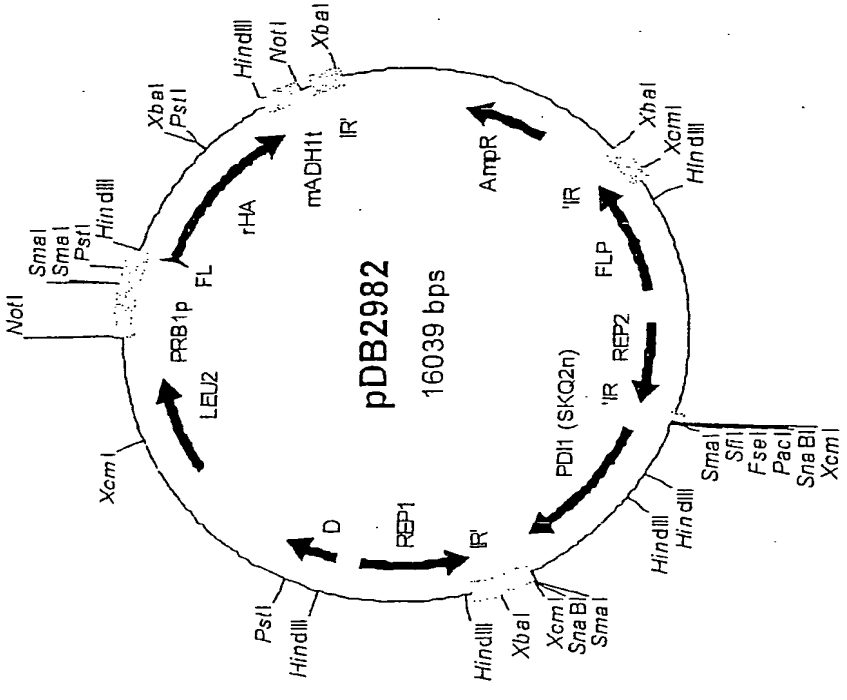


FIGURE 42

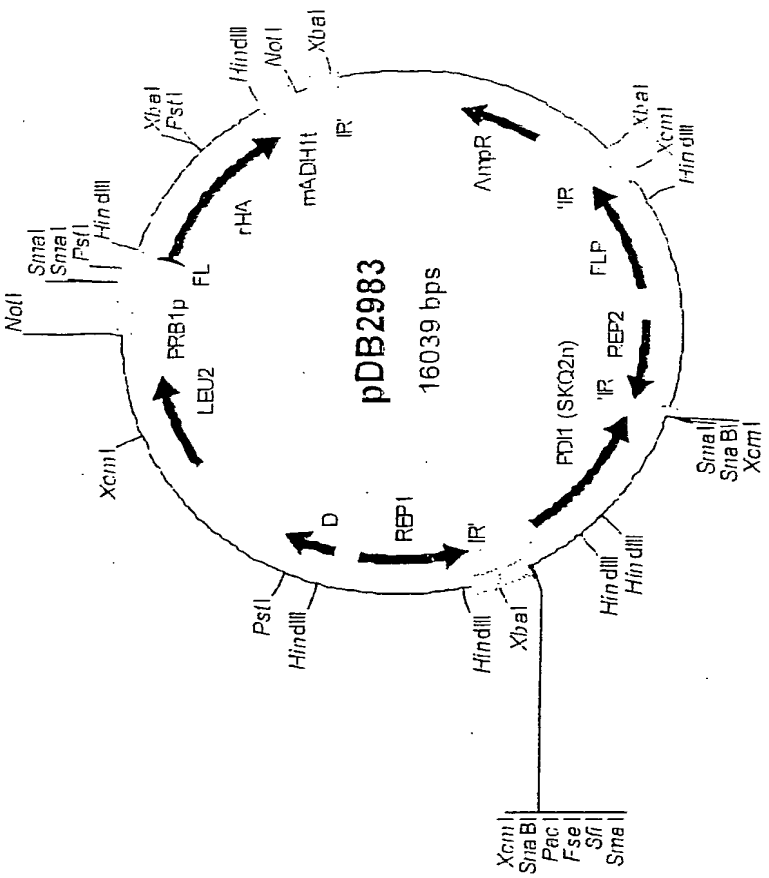


FIGURE 44

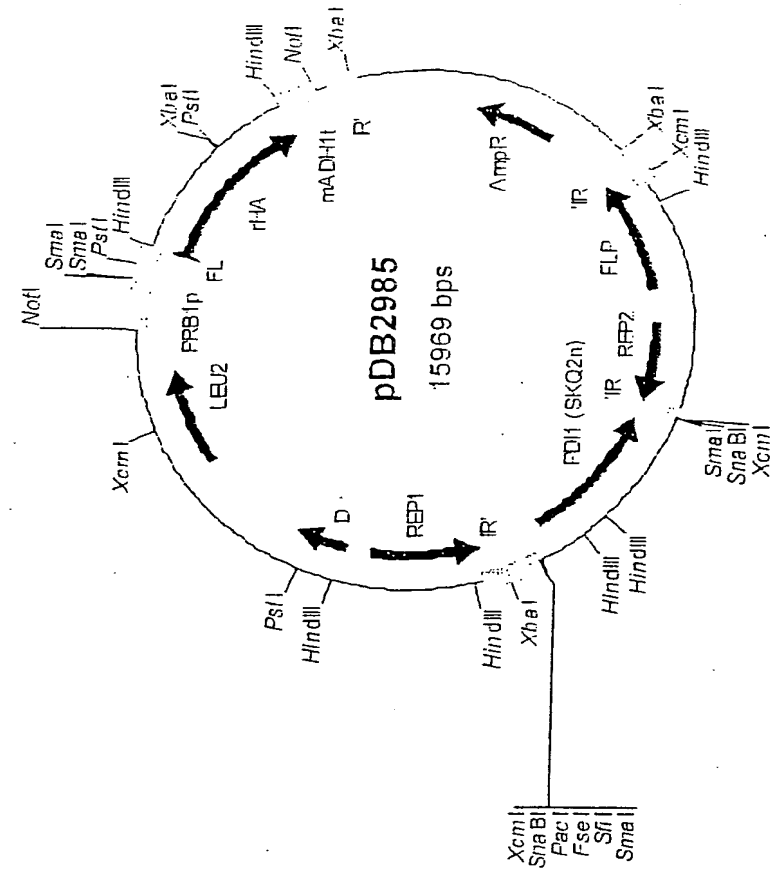


FIGURE 43

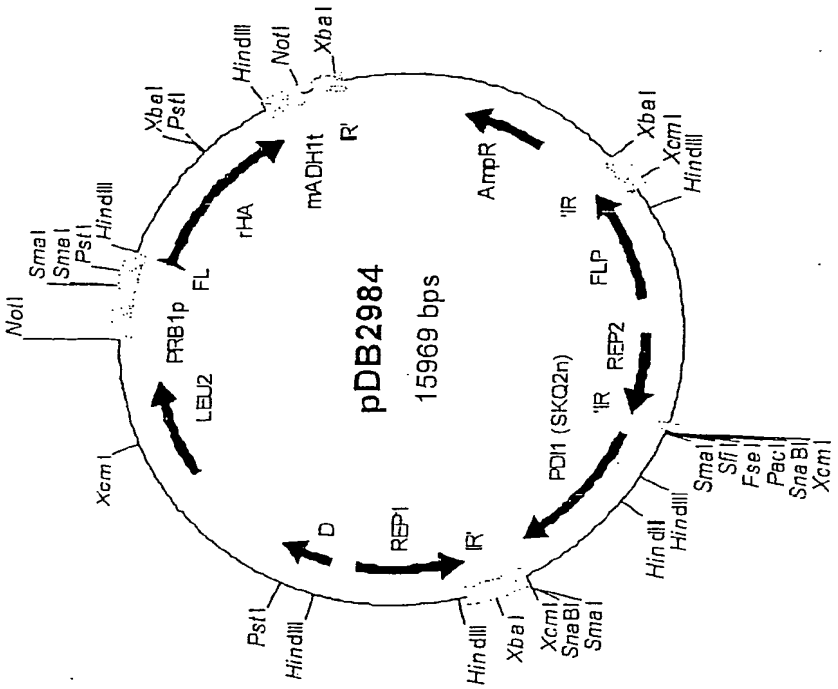


FIGURE 46

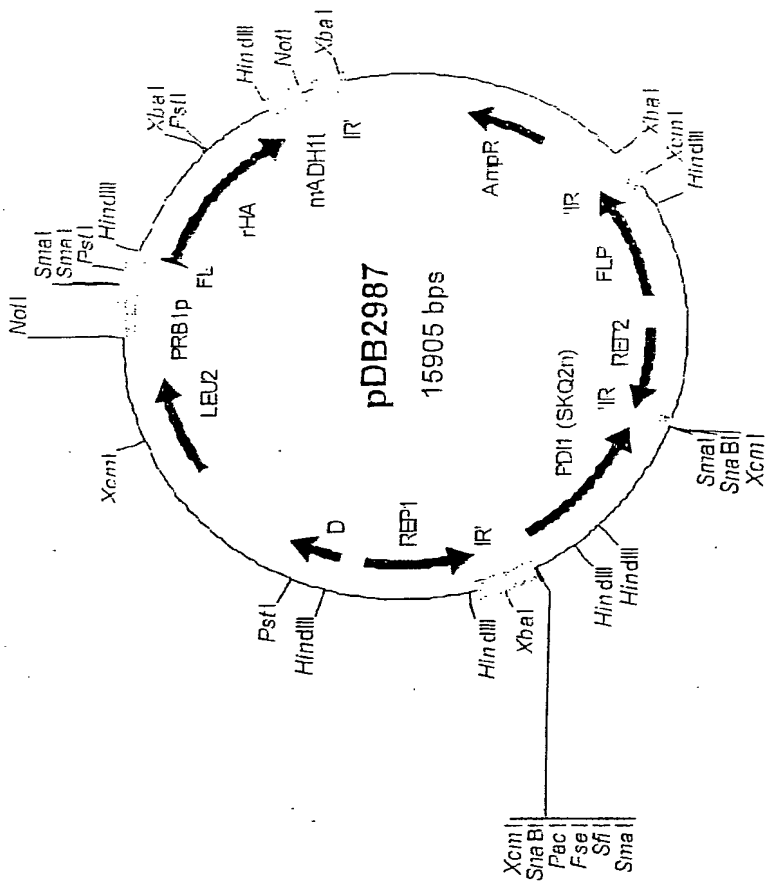


FIGURE 45

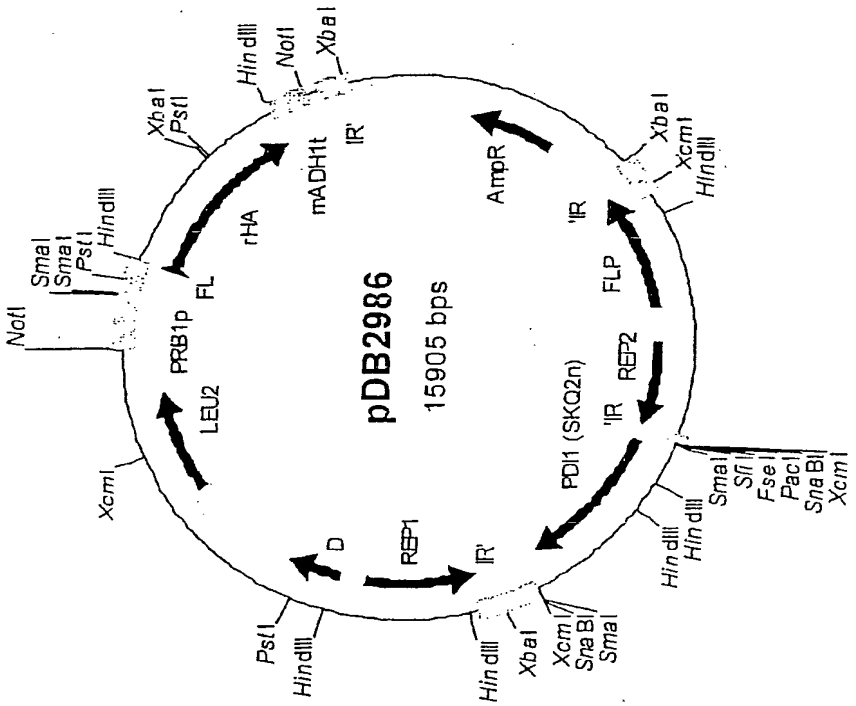


FIGURE 48

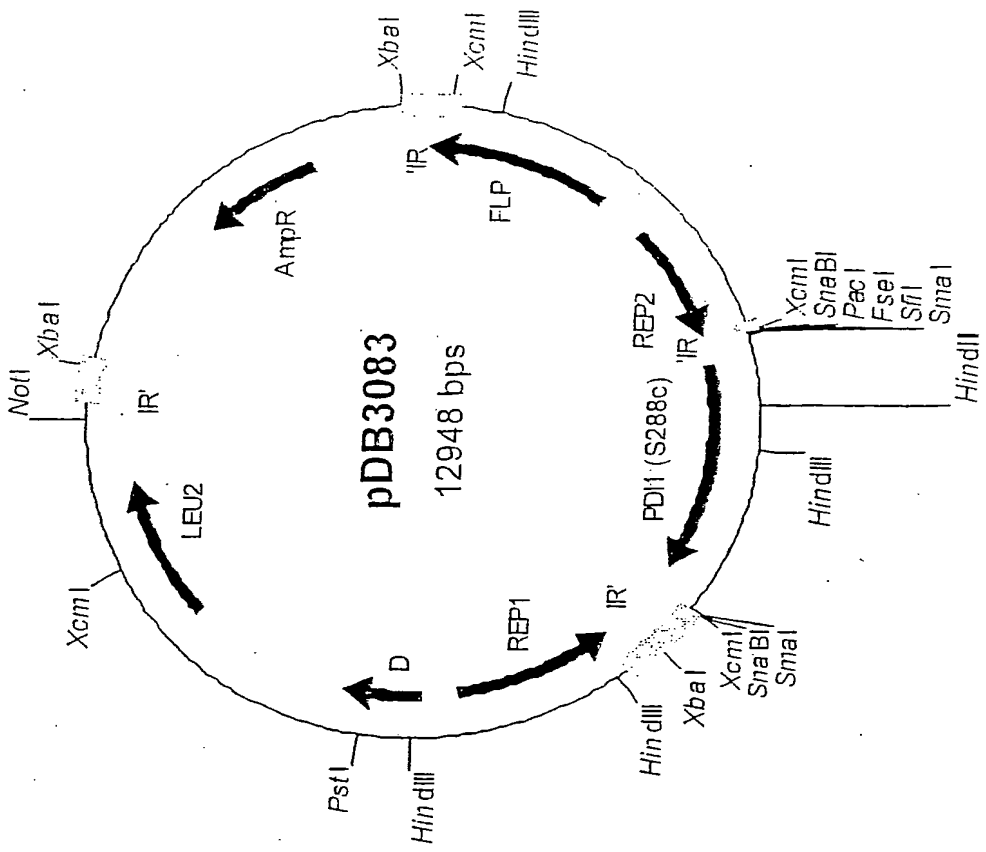


FIGURE 47

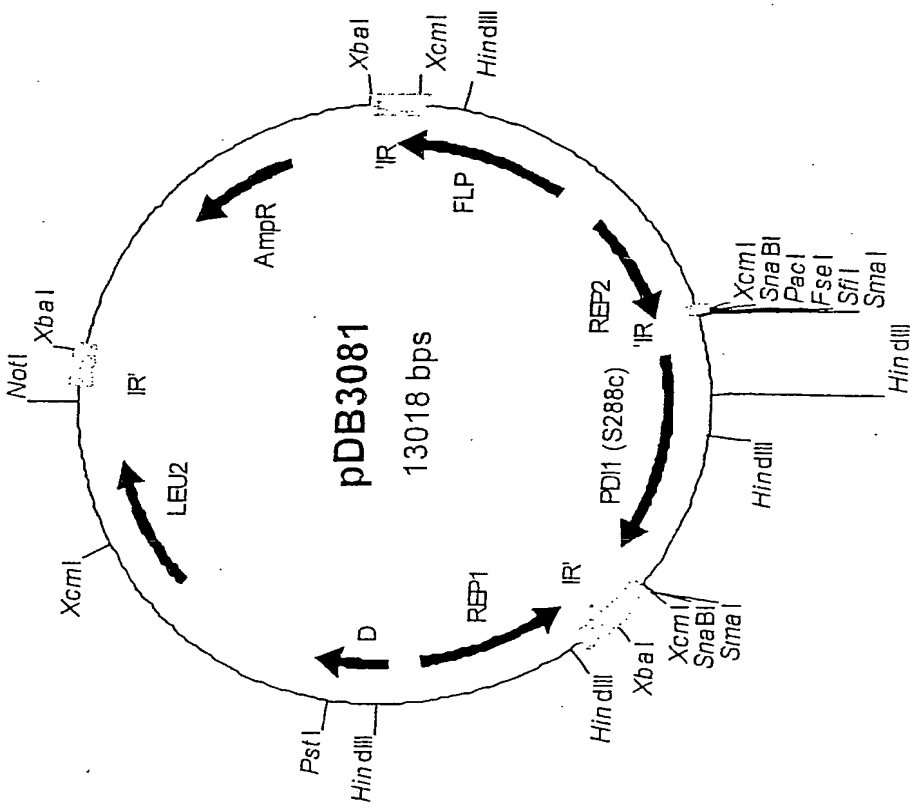


FIGURE 49

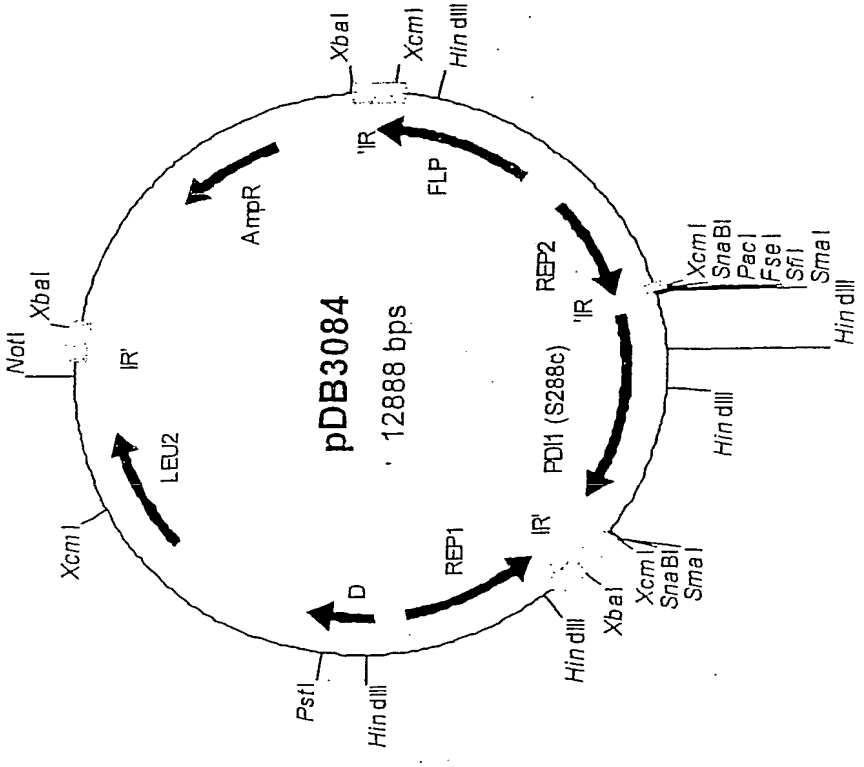


FIGURE 50

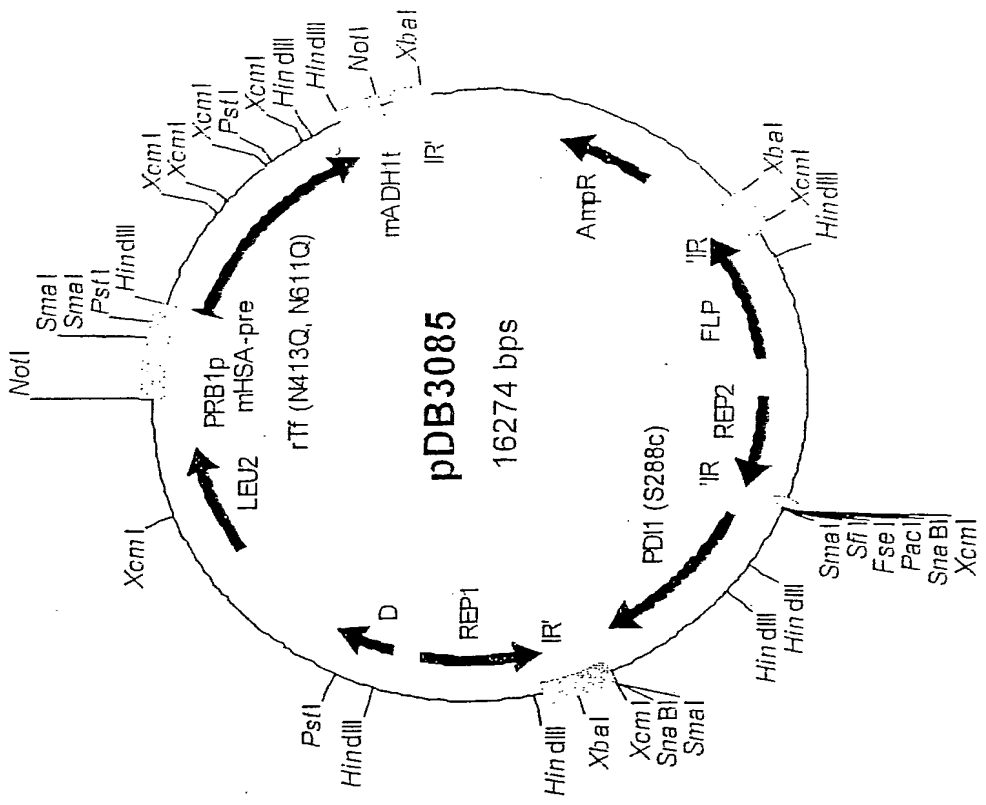


FIGURE 51

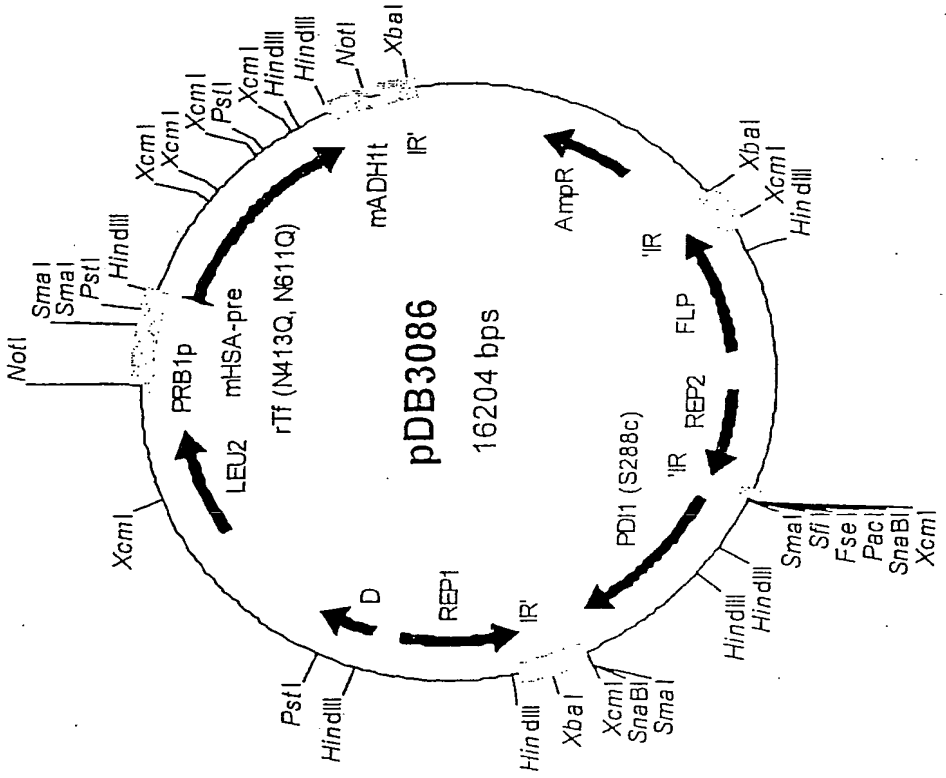


FIGURE 52

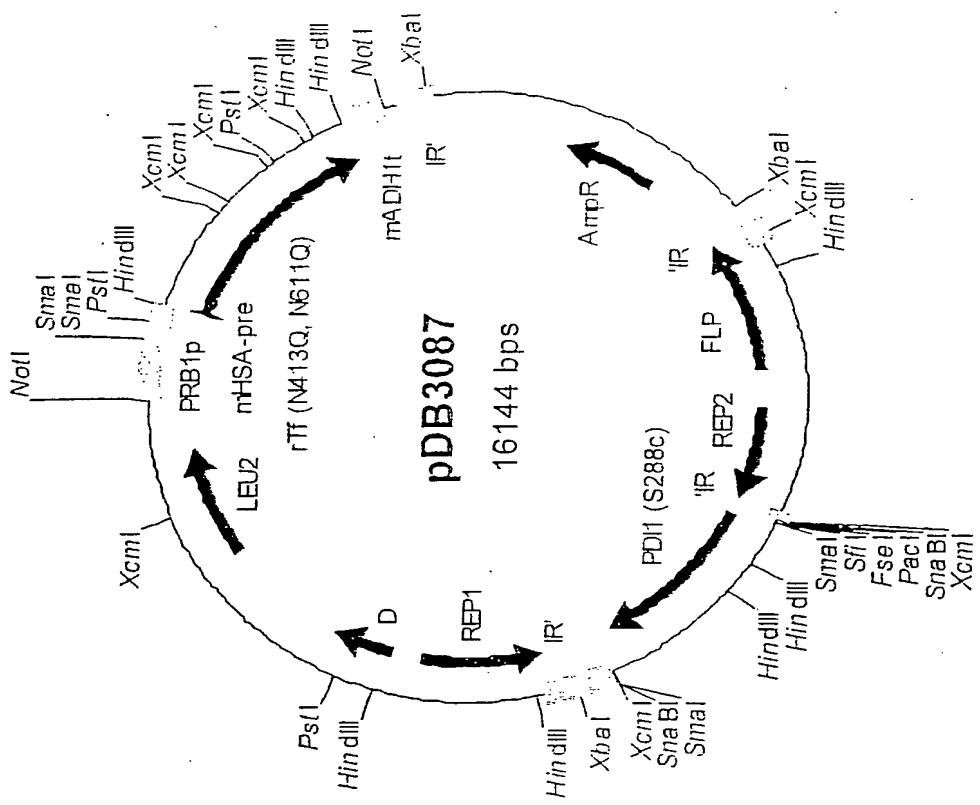
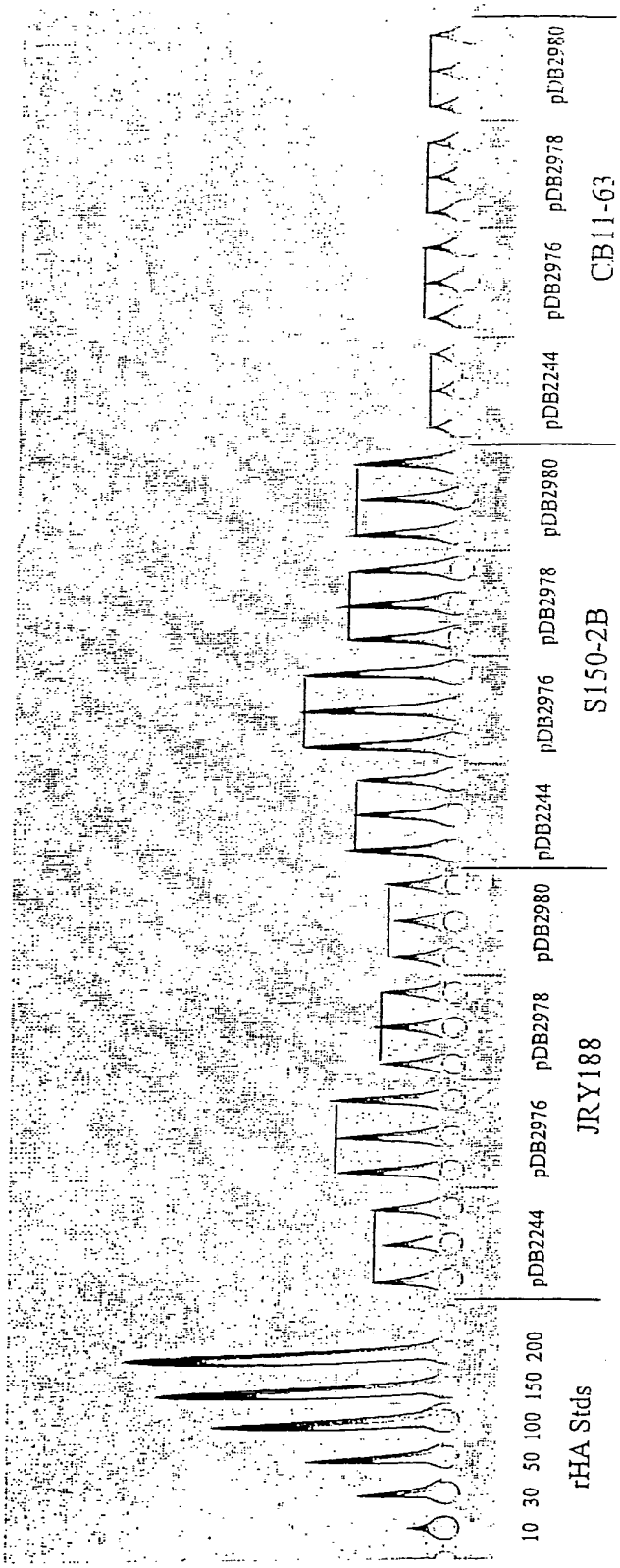


FIGURE 53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pDB2980
S150-2B
pDB2244
pDB2976
pDB2978
pDB2980
CB11-63

FIGURE 54

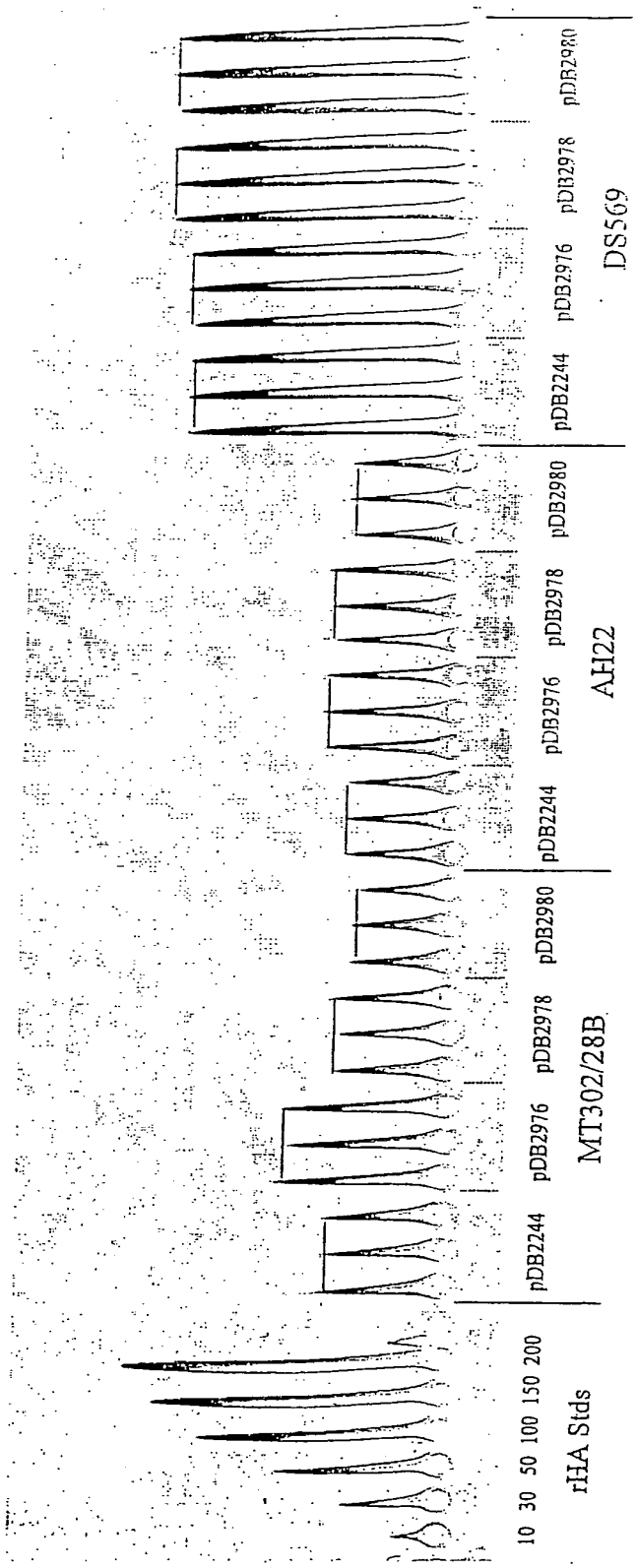

10 30 50 100 150 200
rHA Stds
pDB2244
pDB2976
pDB2978
pDB2980
MT302/28B
pDB2244
pDB2976
pDB2978
pDB2980
AH22
pDB2244
pDB2976
pDB2978
pDB2980
DS569

FIGURE 55

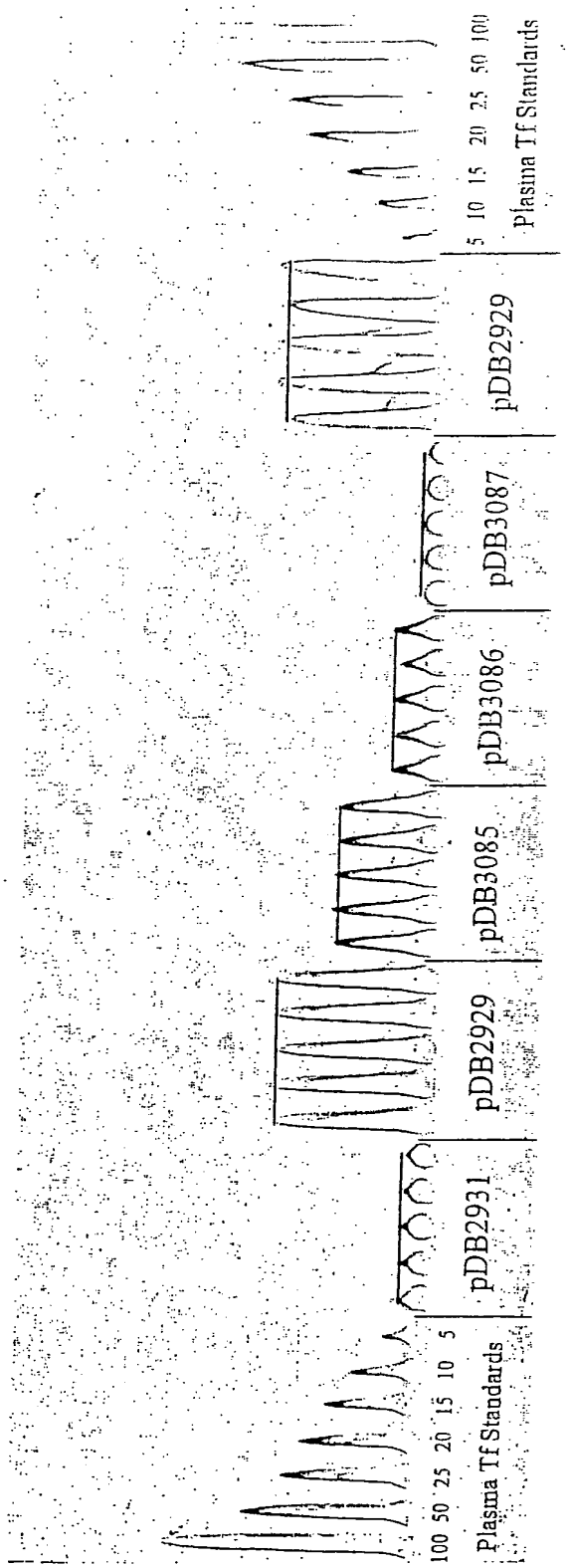

100 50 25 20 15 10 5
Plasma Tf Standards
pDB2931
pDB2929
pDB3085
pDB3086
pDB3087
pDB2929
5 10 15 20 25 50 100
Plasma Tf Standards

Figure 56

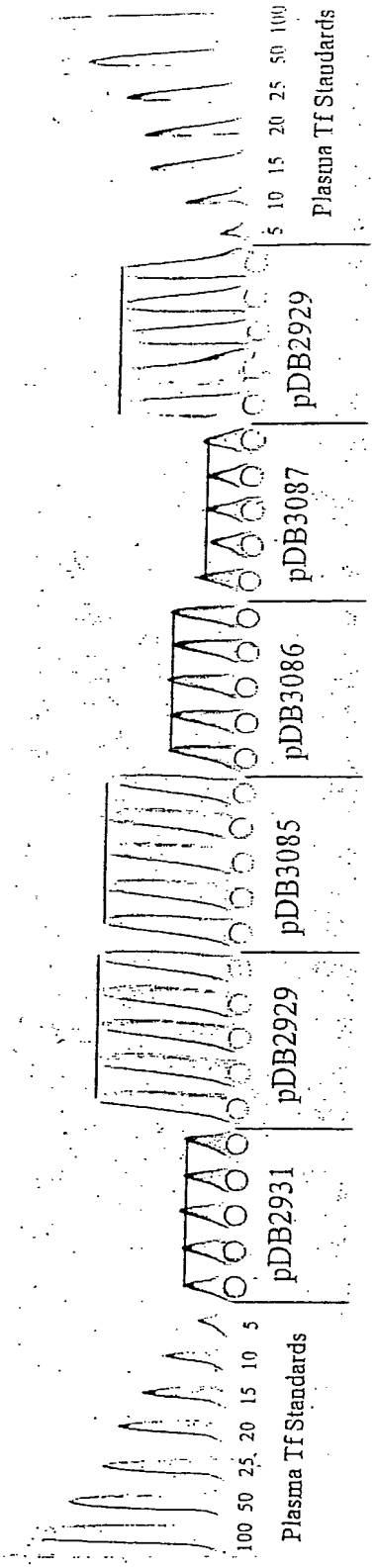

100 50 25 20 15 10 5
Plasma Tf Standards
pDB2931
pDB2929
pDB3085
pDB3086
pDB3087
pDB2929
5 10 15 20 25 50 100
Plasma Tf Standards

FIGURE 57

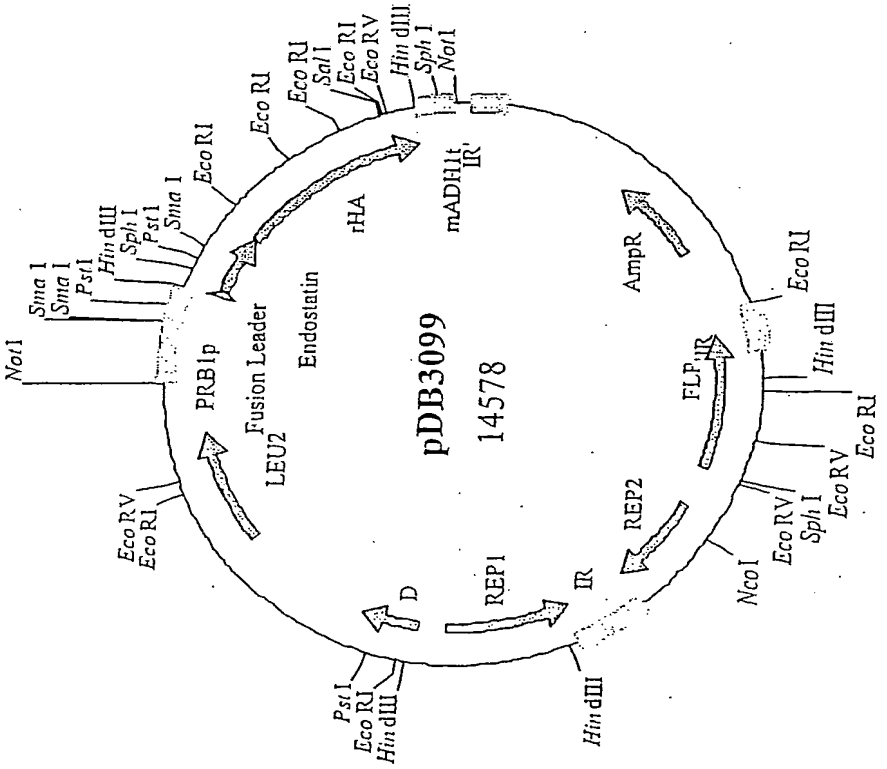


FIGURE 58

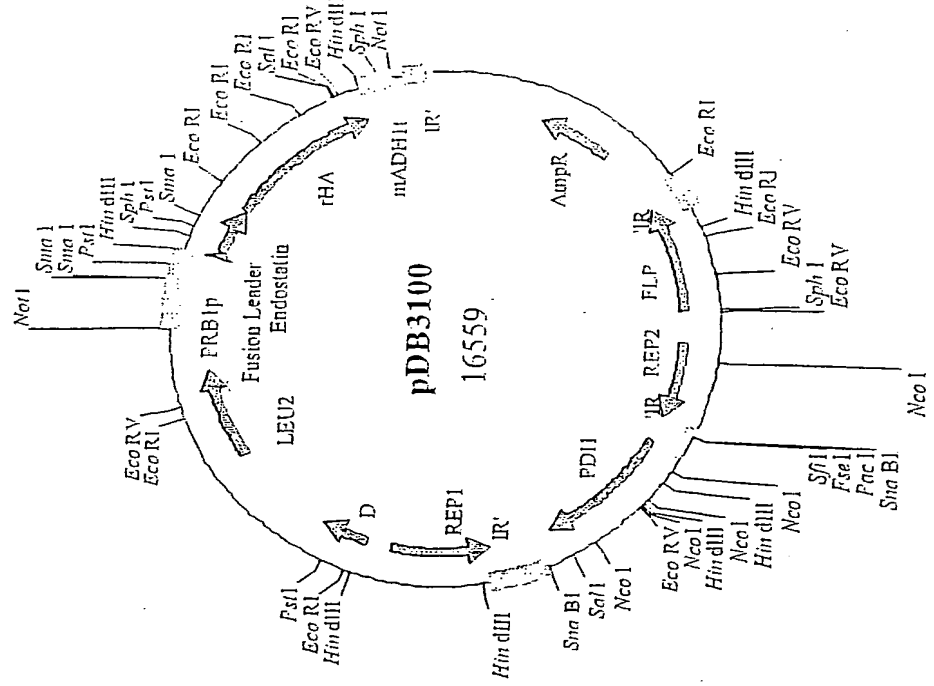


FIGURE 59

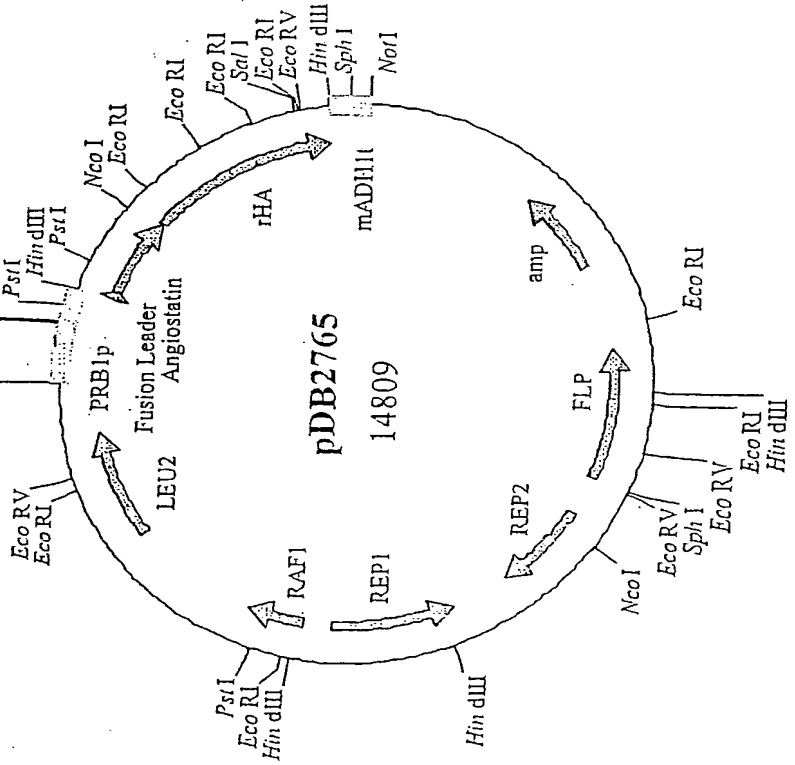


FIGURE 60

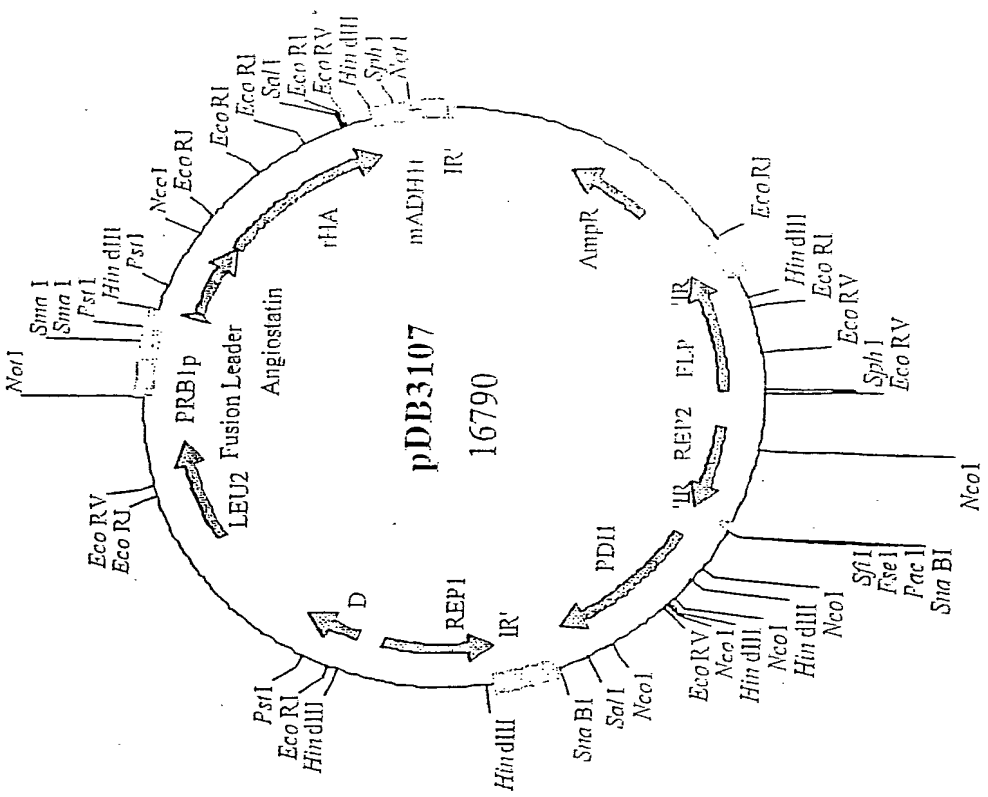


FIGURE 62

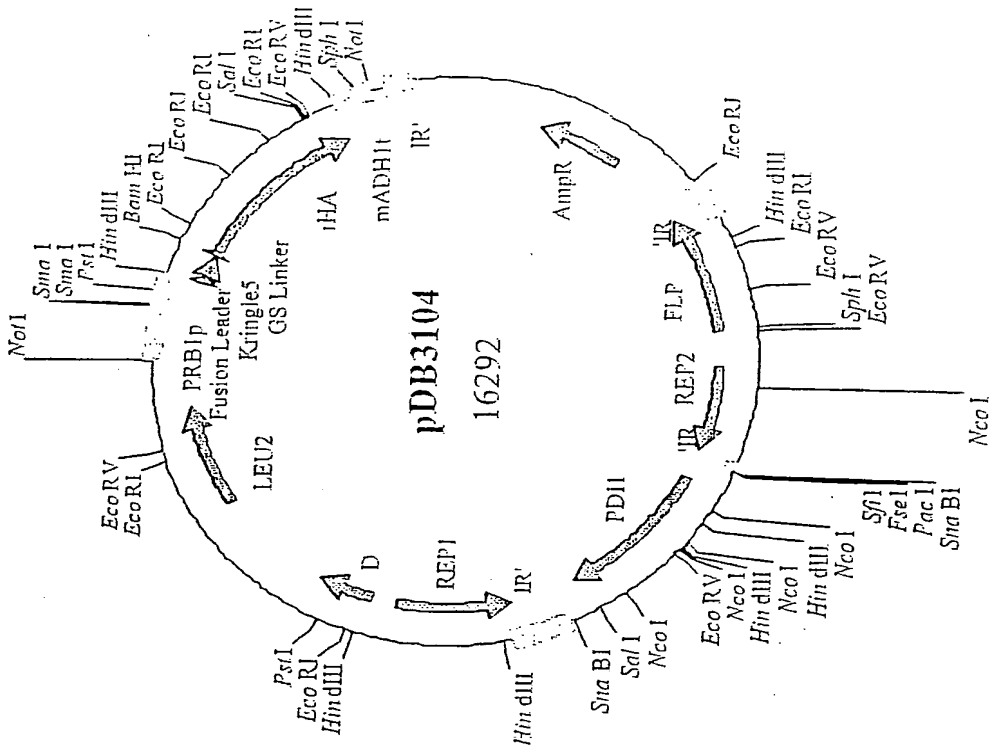


FIGURE 61

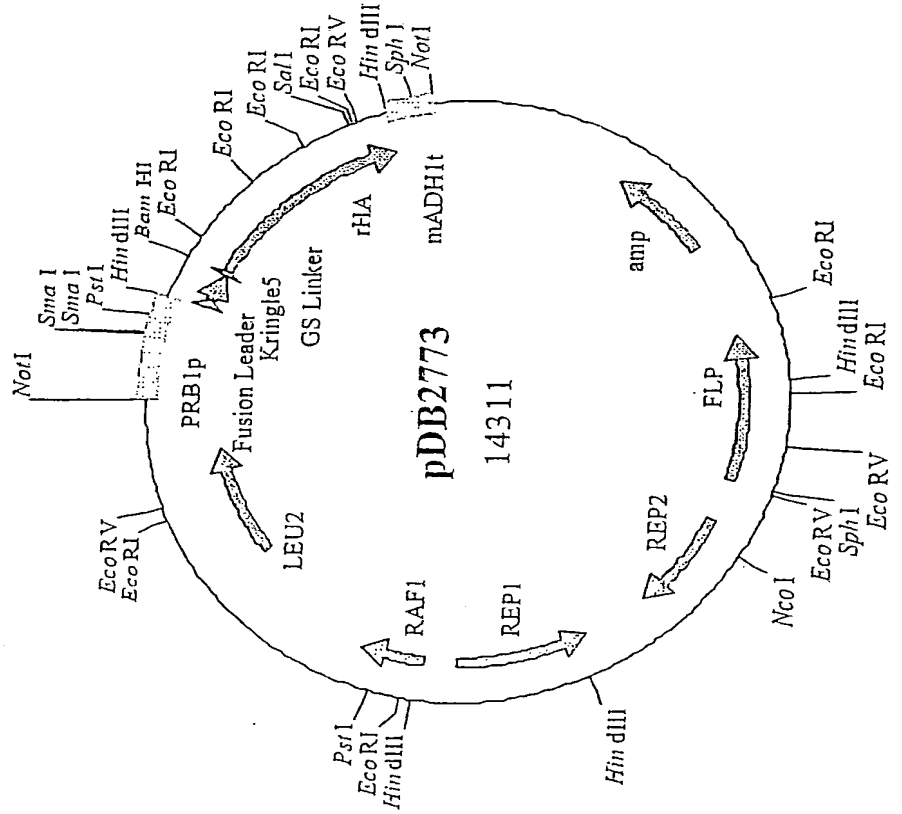


FIGURE 63

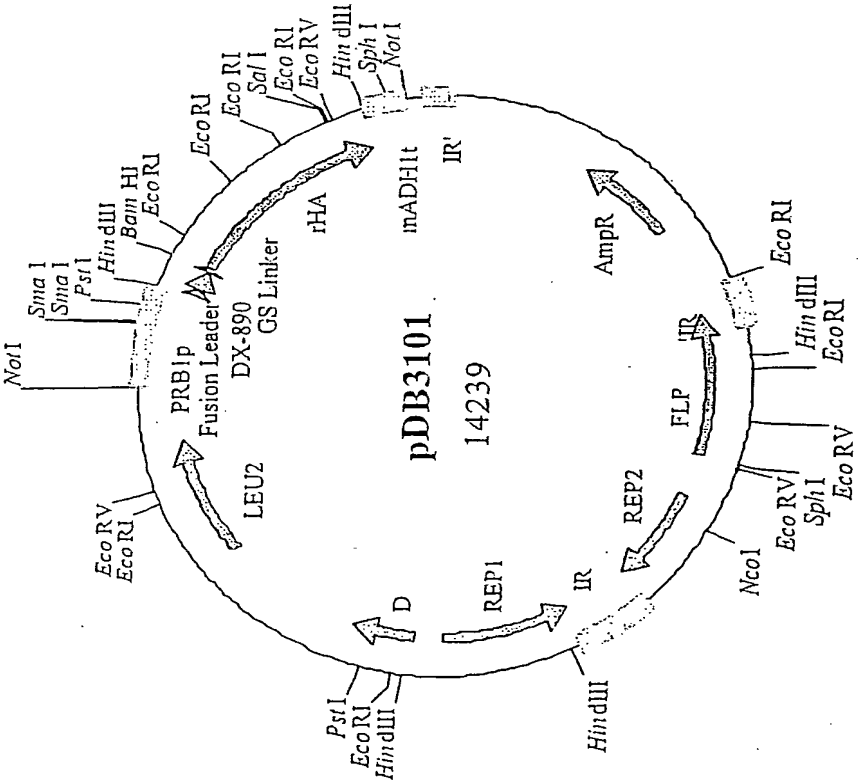


FIGURE 64

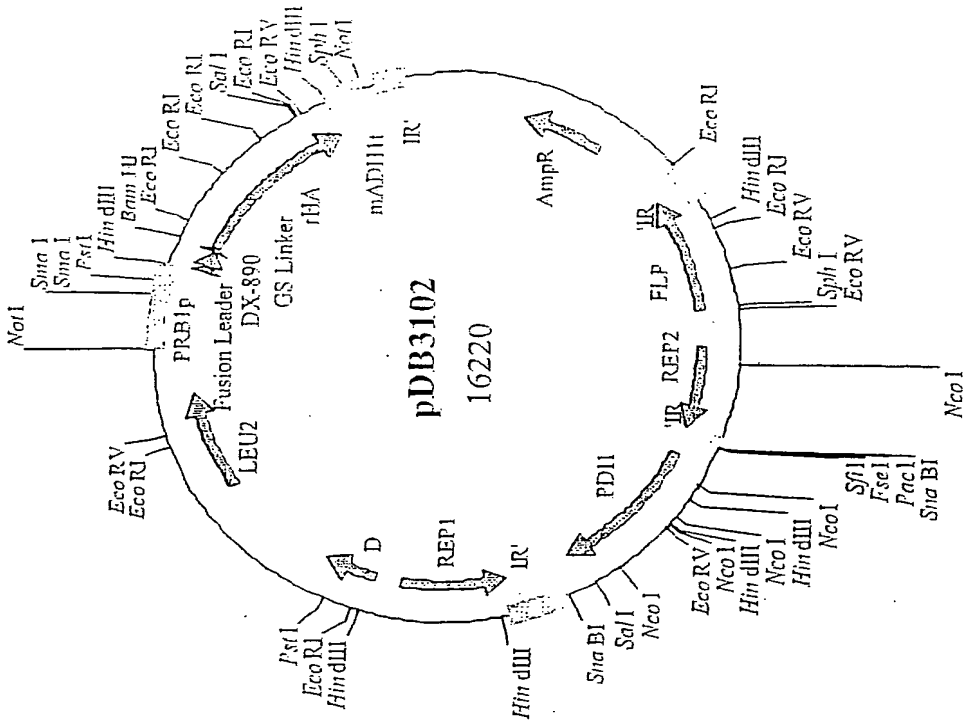


FIGURE 65

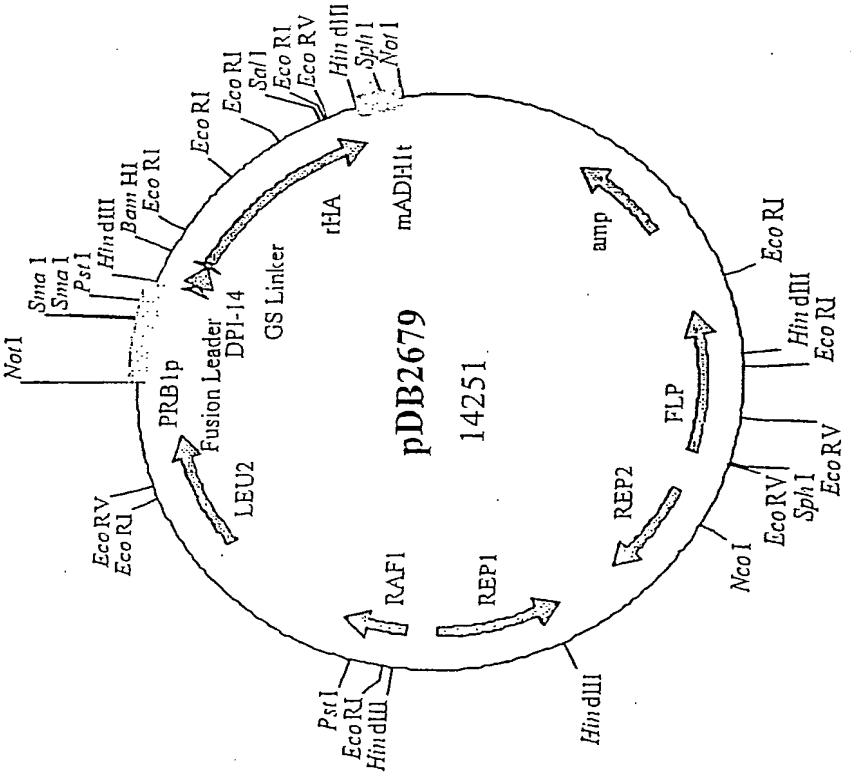


FIGURE 66

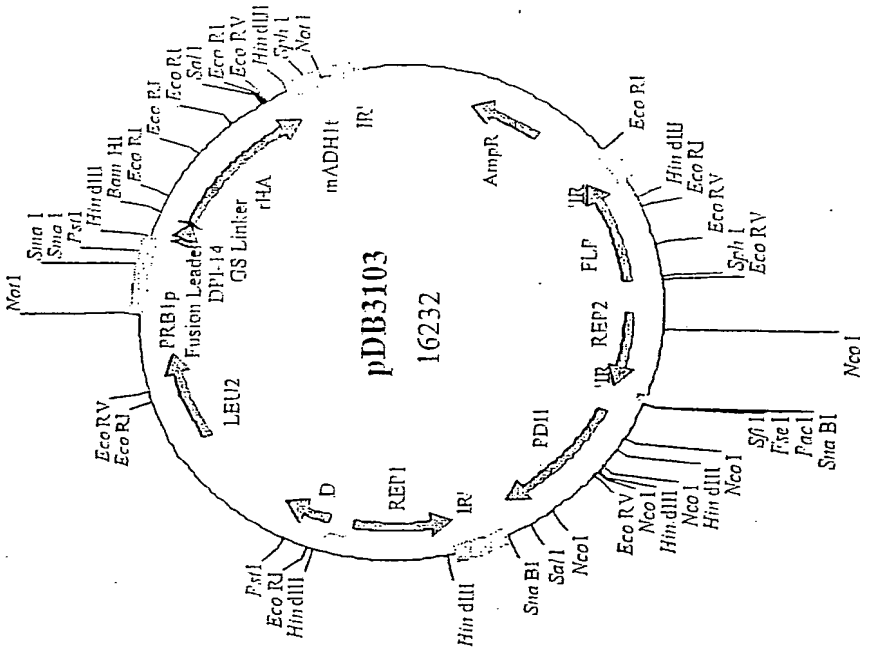


FIGURE 67

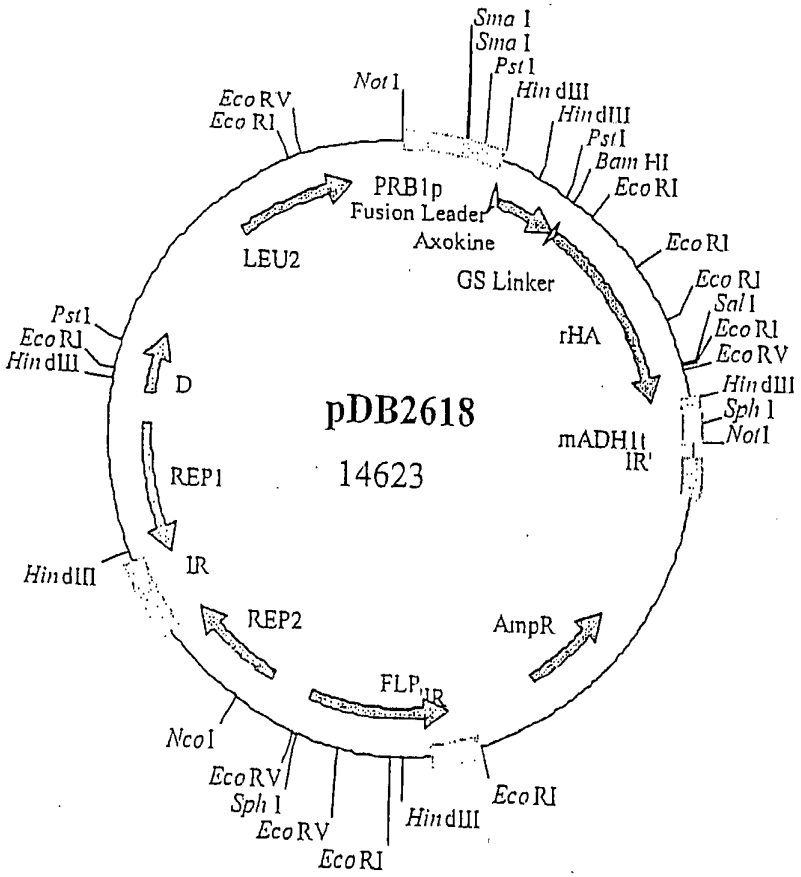


FIGURE 68

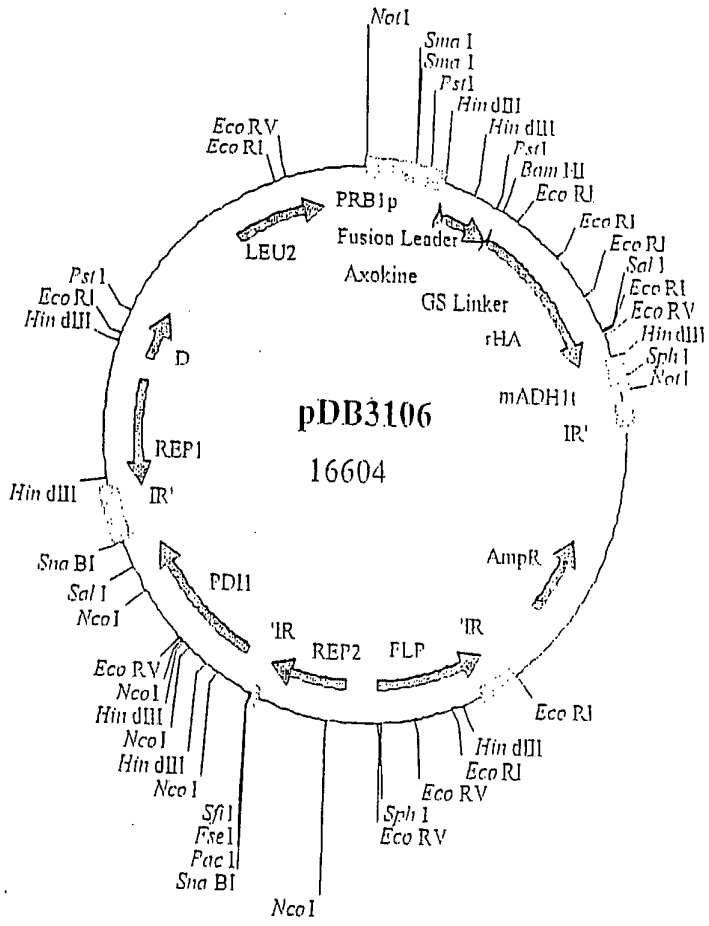


FIGURE 69

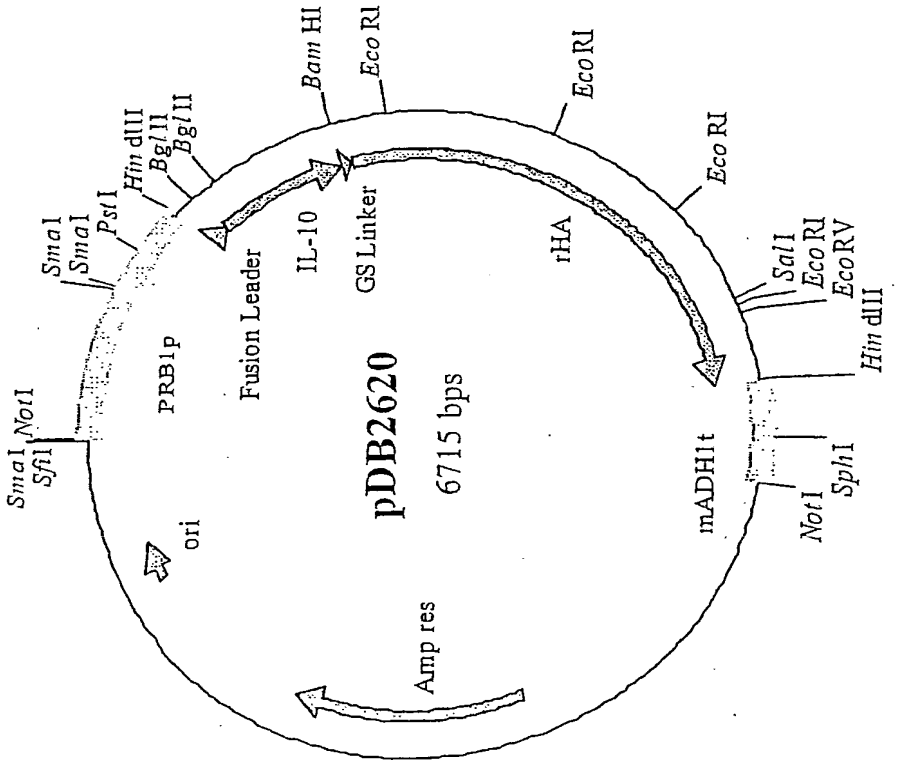


FIGURE 70

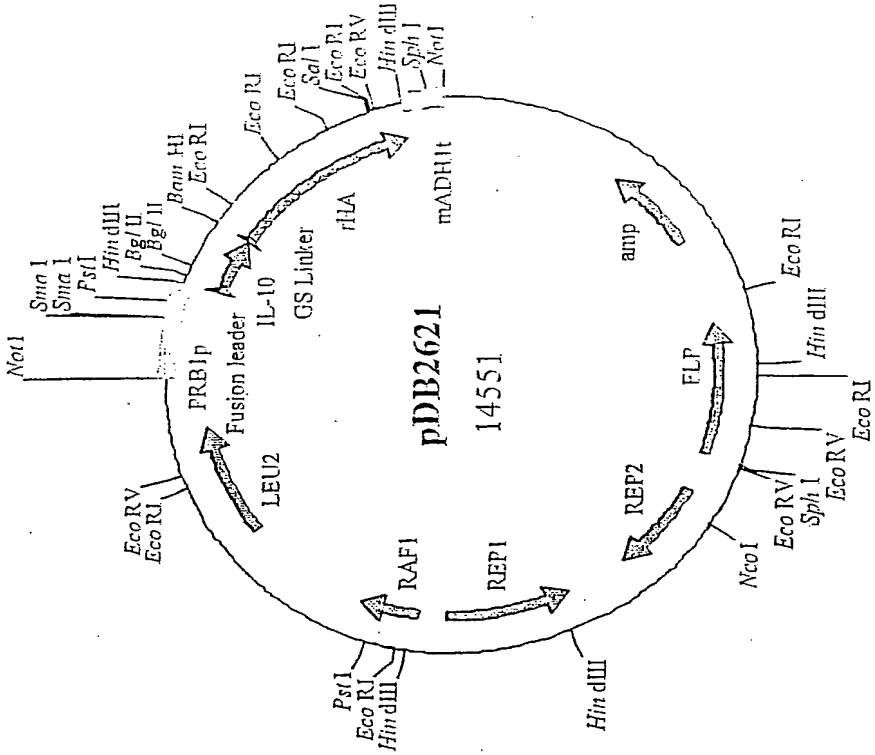


FIGURE 71

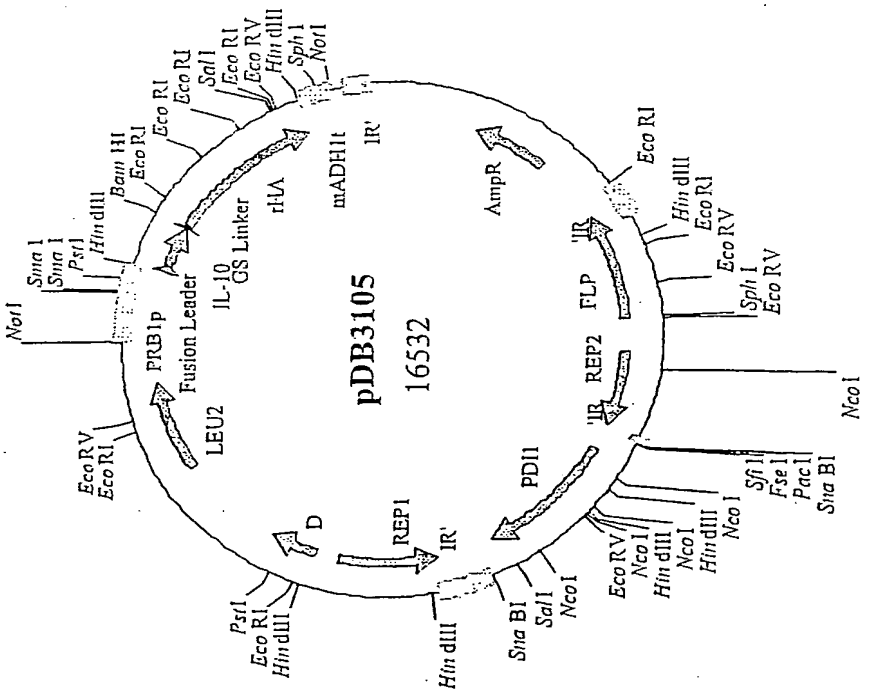
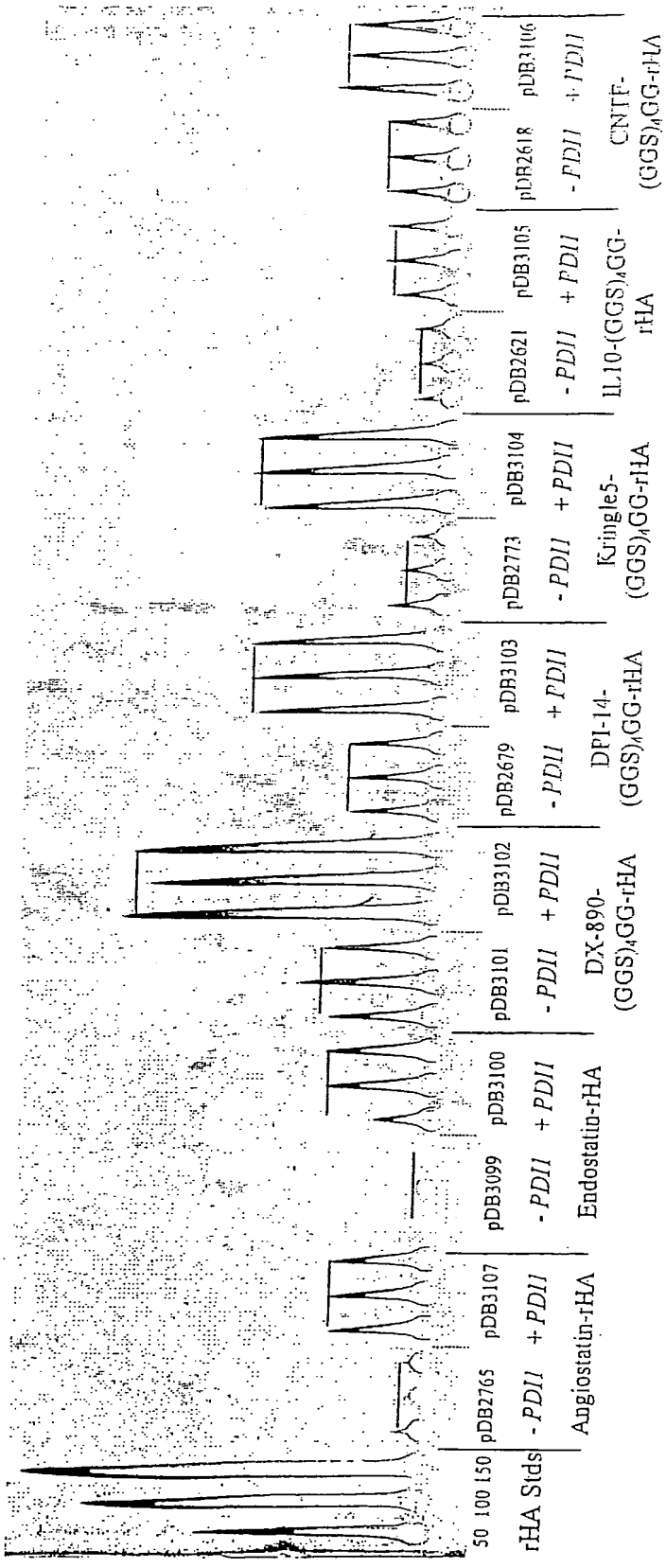

pDB3105
16532
PRB1p
Fusion Leader
IL-10
GS Linker
rHA
mADH1t
IR'
AmpR
'IR
FLP
REP2
'IR
PDI1
REP1
IR'
D
LEU2
Not I
Sma 1
Sma 1
Pst I
Hin dIII
Bam HI
Eco RI
Eco RI
Eco RI
Sal I
Eco RI
Eco RV
Hin dIII
Sph I
Not I
Eco RV
Eco RI
Pst I
Eco RI
Hin dIII
Hin dIII
Sna BI
Sal I
Nco I
Eco RV
Nco I
Hin dIII
Nco I
Hin dIII
Nco I
Sfi I
Fse I
Pac I
Sna BI
Nco I
Eco RI
Hin dIII
Eco RI
Eco RV
Sph I
Eco RV

FIGURE 72


50 100 150
rHA Stds
pDB2765
pDB3107
- PDI1 + PDI1
Angiostatin-rHA
pDB3099
pDB3100
- PDI1 + PDI1
Endostatin-rHA
pDB3101
pDB3102
- PDI1 + PDI1
DX-890-(GGS)4GG-rHA
pDB2679
pDB3103
- PDI1 + PDI1
DPI-14-(GGS)4GG-rHA
pDB2773
pDB3104
- PDI1 + PDI1
Kringle5-(GGS)4GG-rHA
pDB2621
pDB3105
- PDI1 + PDI1
IL10-(GGS)4GG-rHA
pDB2618
pDB3106
- PDI1 + PDI1
CNTF-(GGS)4GG-rHA

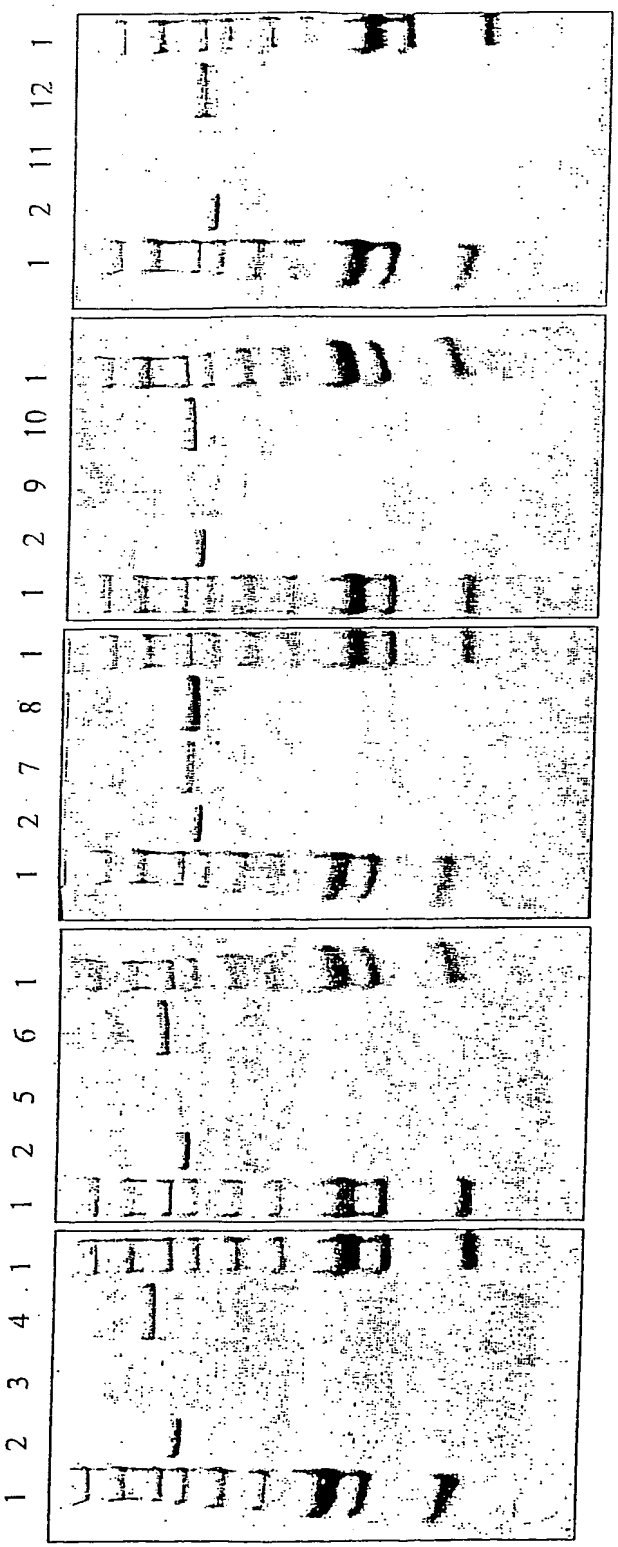


FIGURE 73

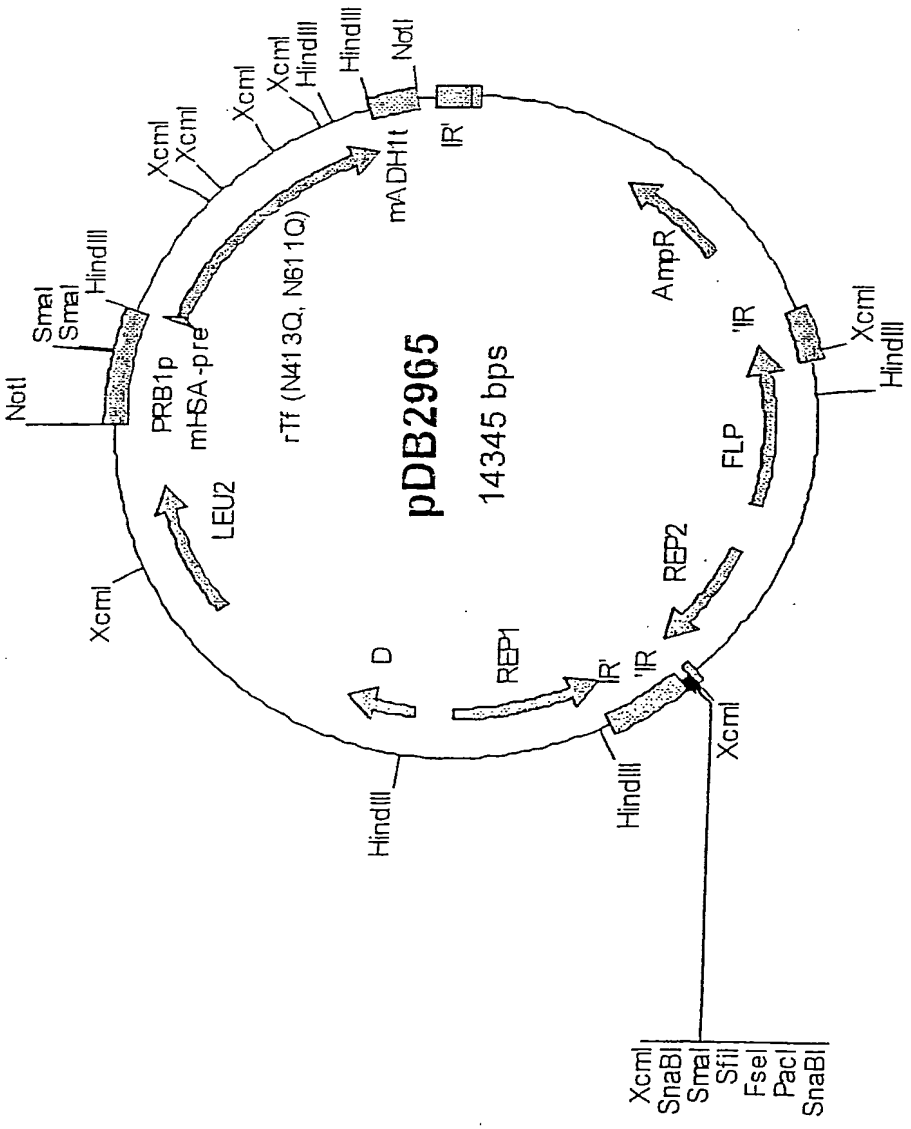

pDB2965
14345 bps
rTf (N413Q, N611Q)
mHSA-pre
PRB1p
LEU2
mADH1t
AmpR
FLP
REP2
REP1
D
IR'
'IR
Xcml
HindIII
NotI
SmaI
SnaBI
SfiI
FseI
PacI

FIGURE 74

FIGURE 75

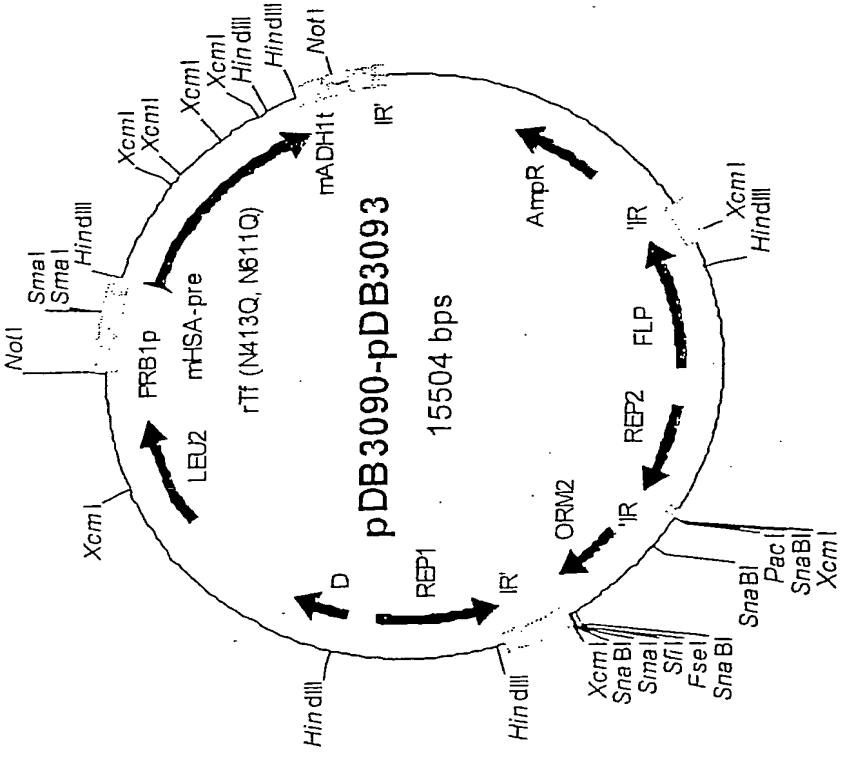
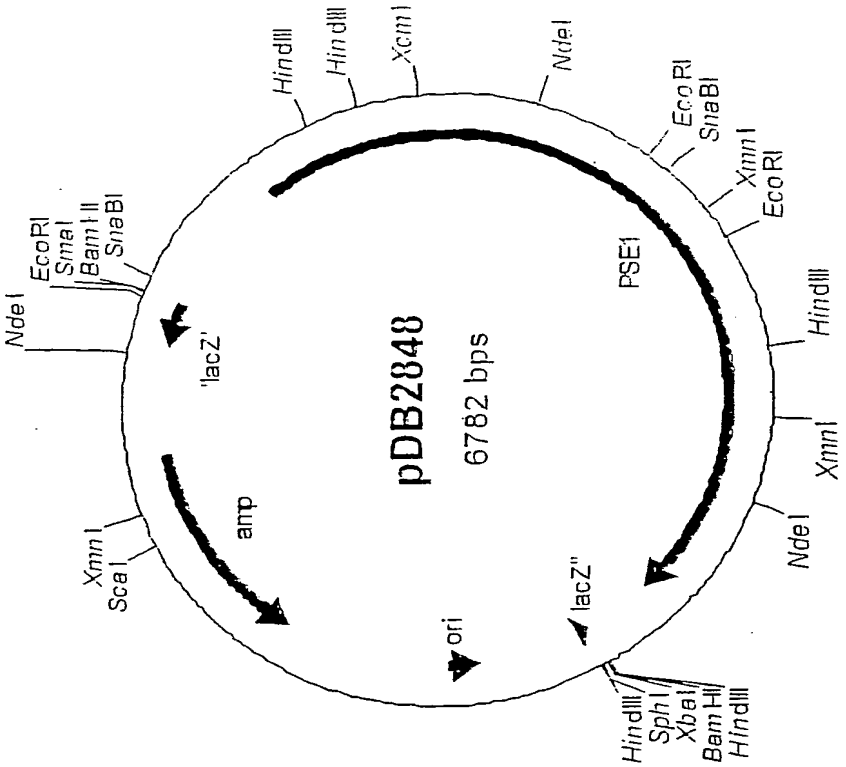


FIGURE 77



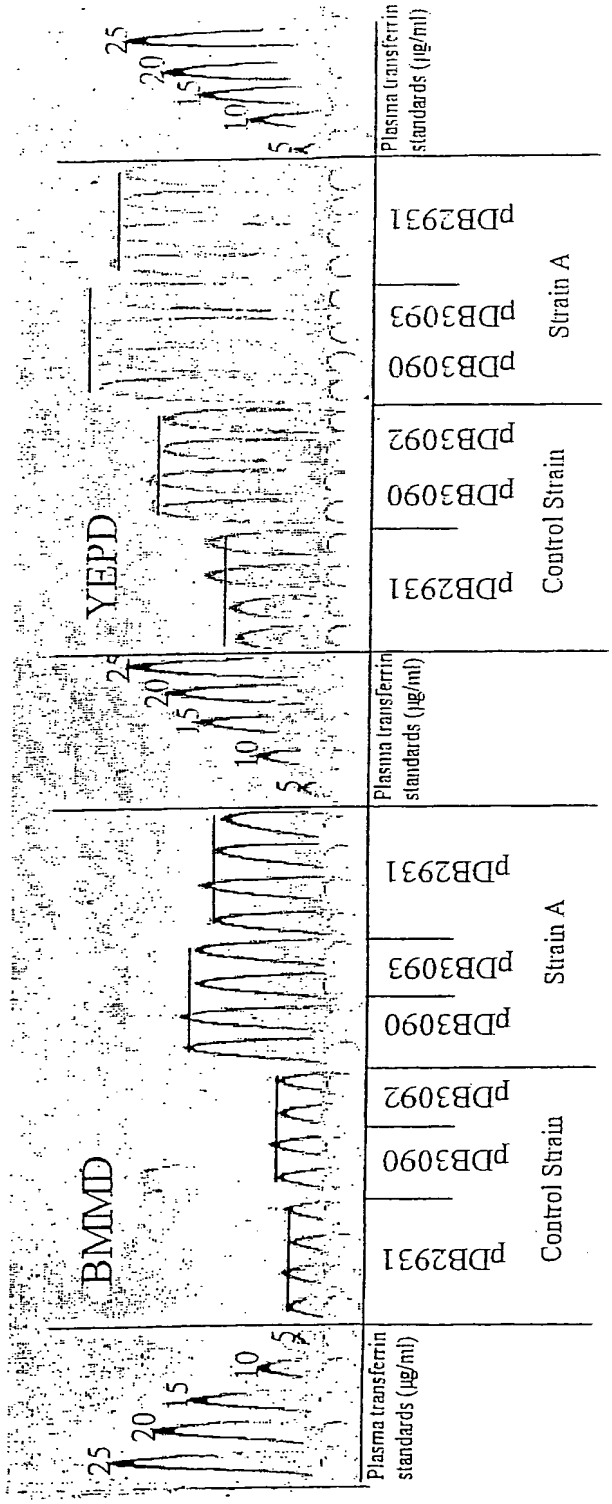

BMMD
YEPD
Plasma transferrin standards (µg/ml)
25
20
15
10
5
pDB2931
pDB3090
pDB3092
pDB3090
pDB3093
pDB2931
Control Strain
Strain A

Figure 76

FIGURE 78

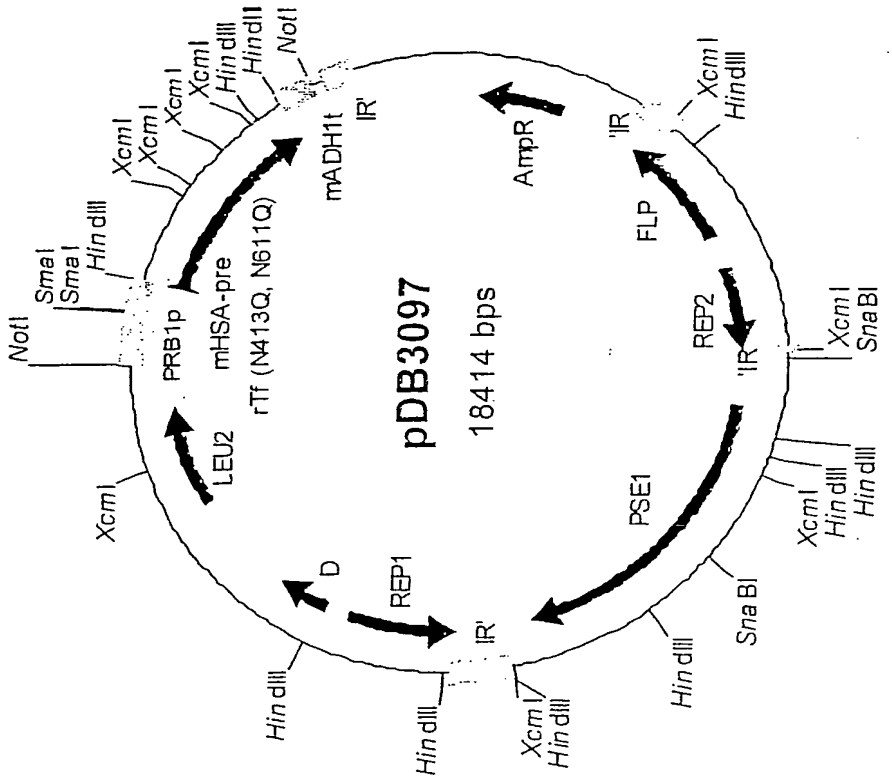
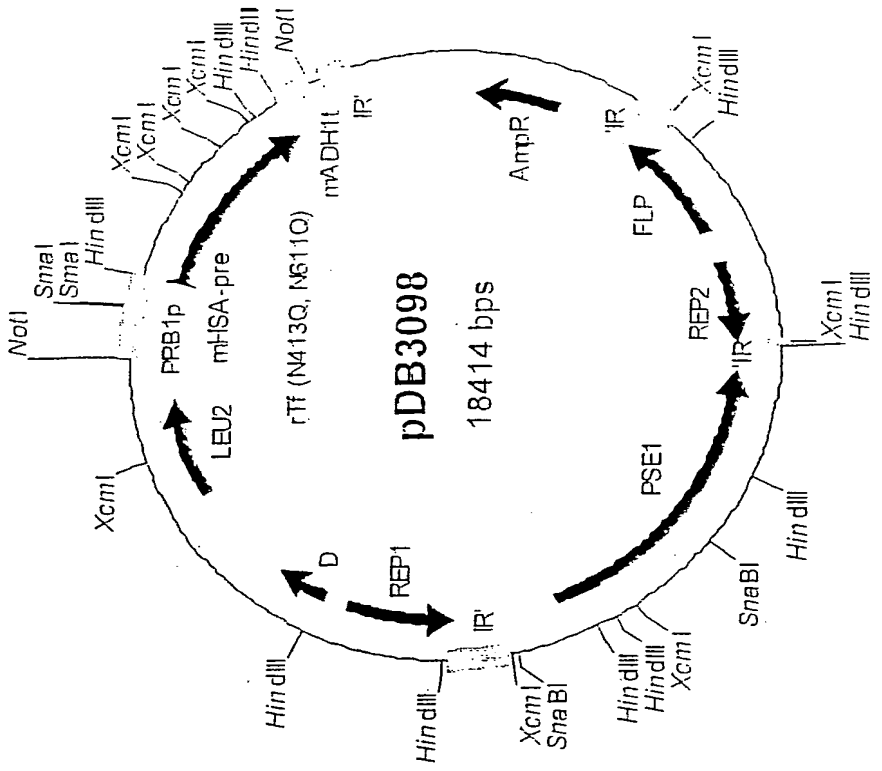


FIGURE 79



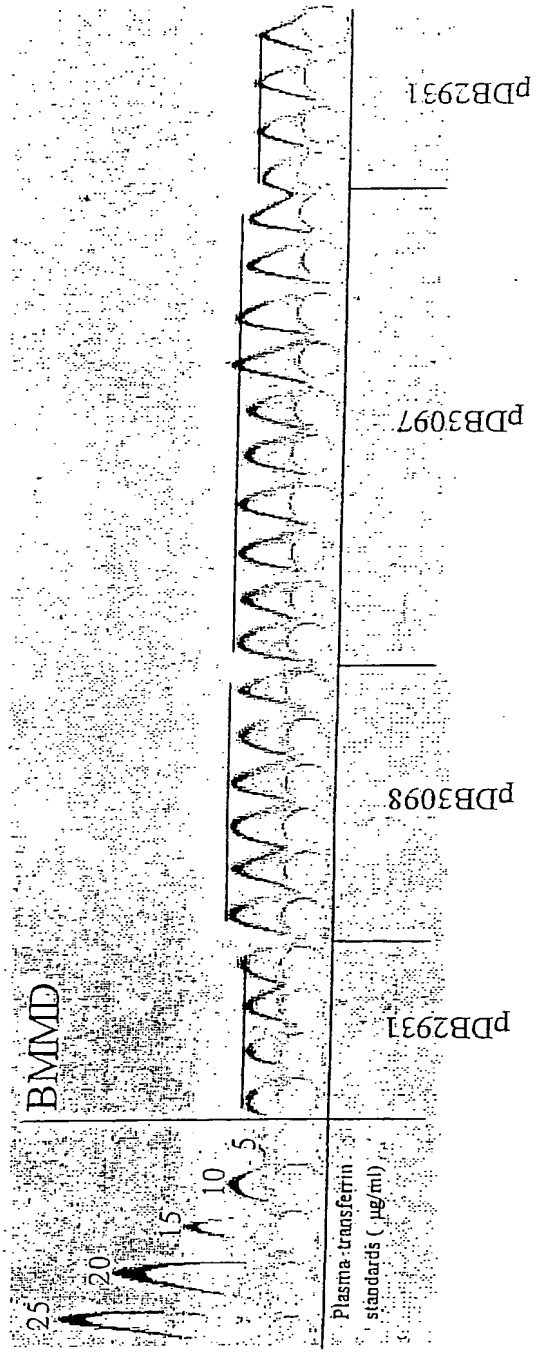

pDB2931
pDB3097
pDB3098
pDB2931
BMMD
25
20
15
10
5
Plasma transferrin standards (µg/ml)

Figure 80

FIGURE 82

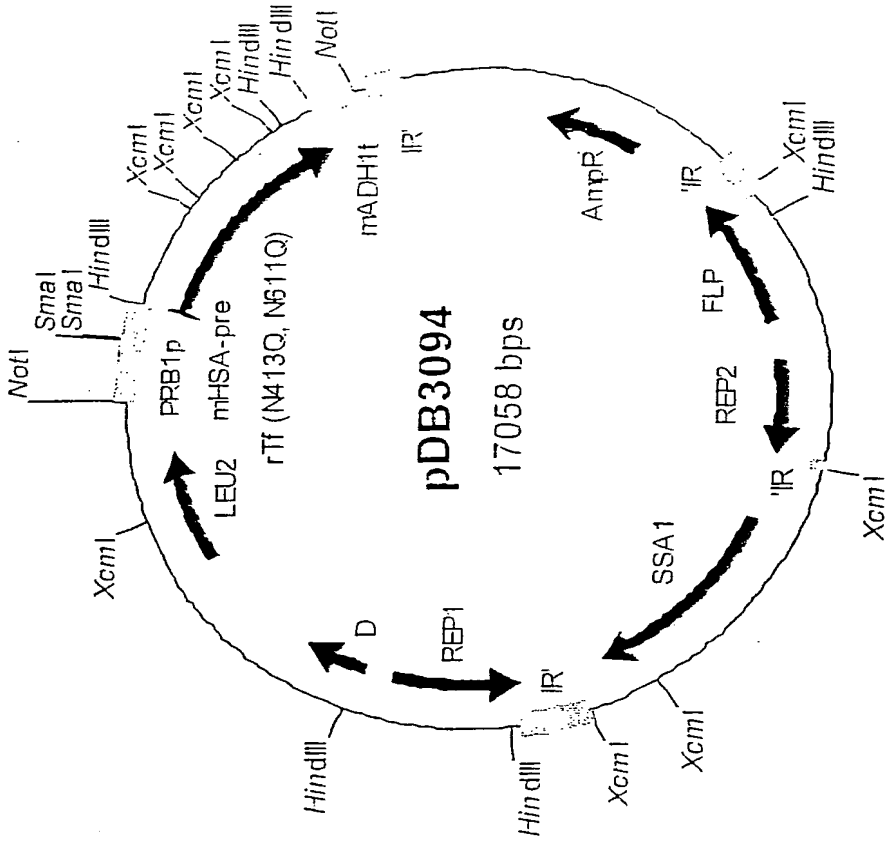


FIGURE 81

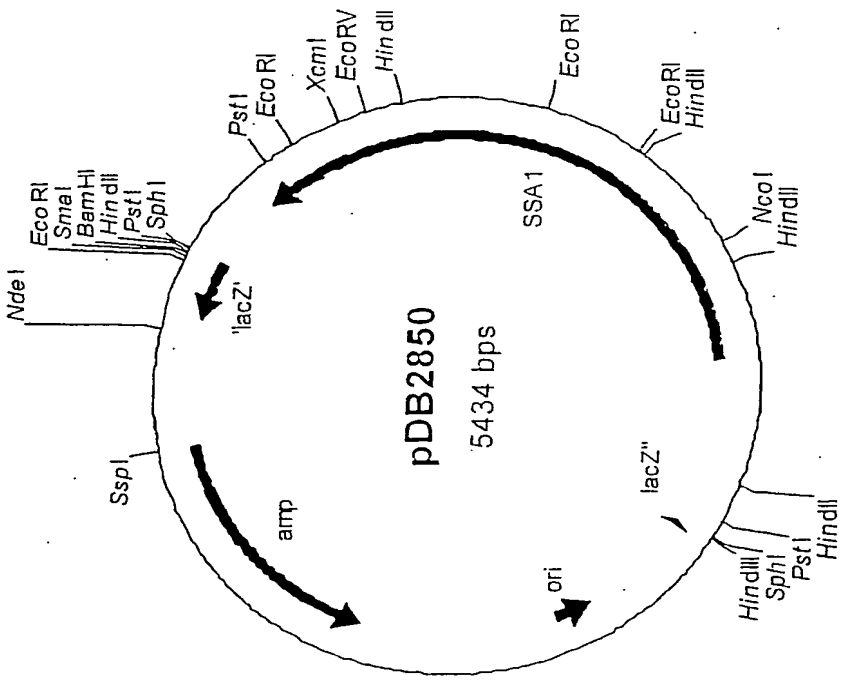


FIGURE 83

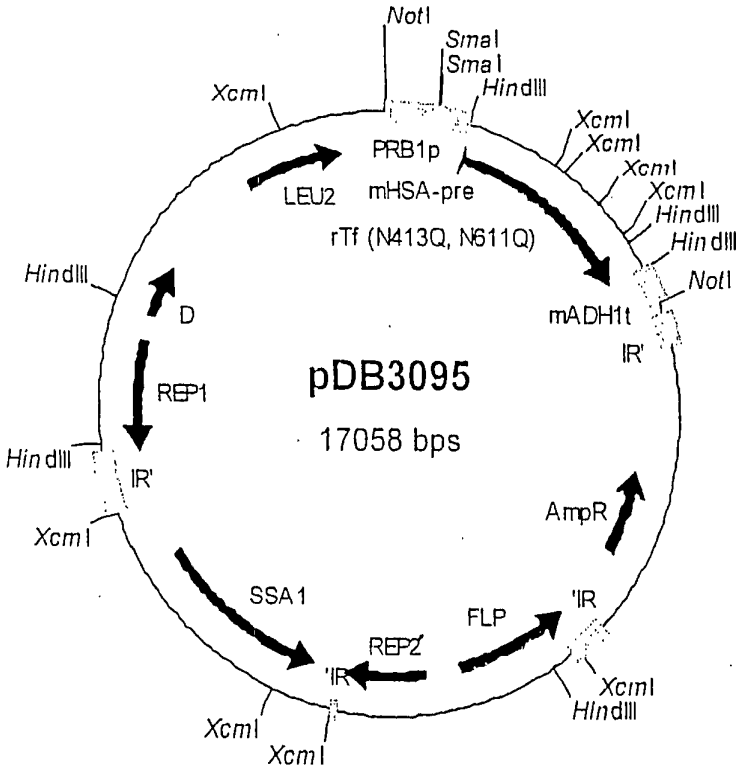
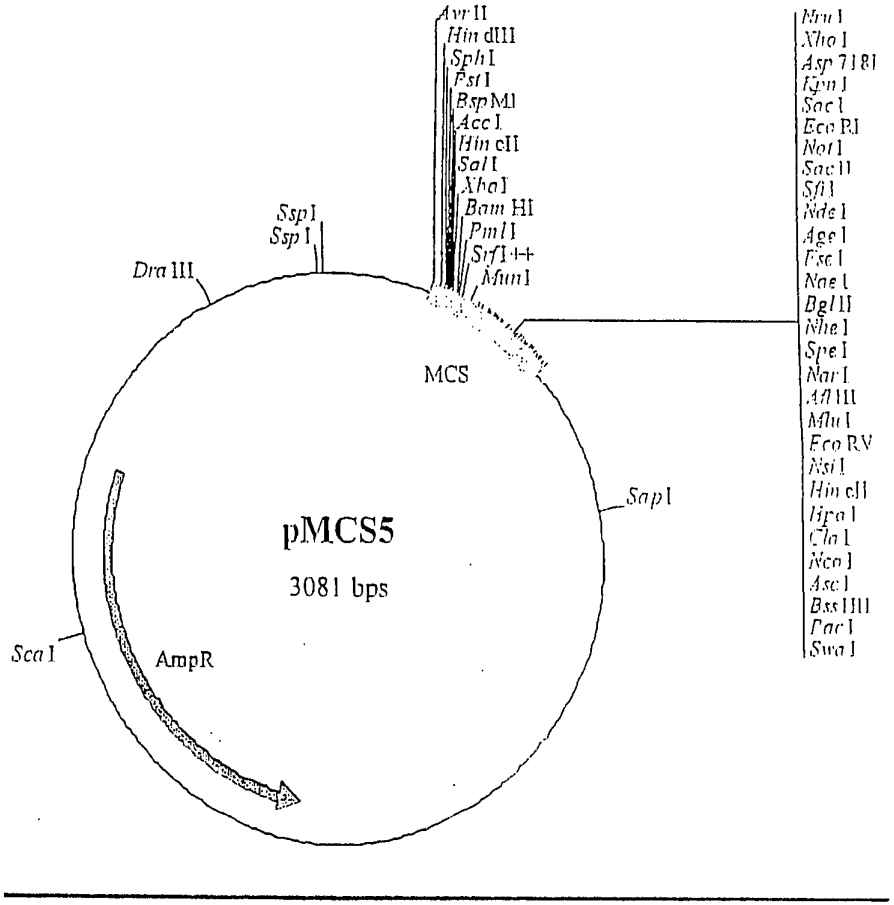


FIGURE 85



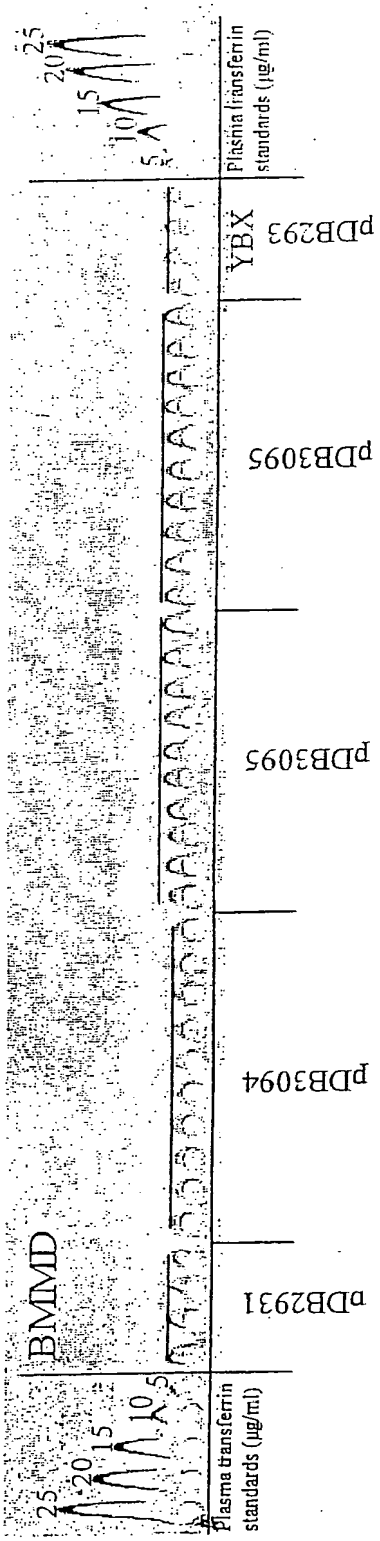

Plasma transferrin standards (µg/ml)
pDB2931
BMMD
pDB3094
pDB3095
pDB3095
YBX
pDB293
Plasma transferrin standards (µg/ml)

Figure 84

FIGURE 86

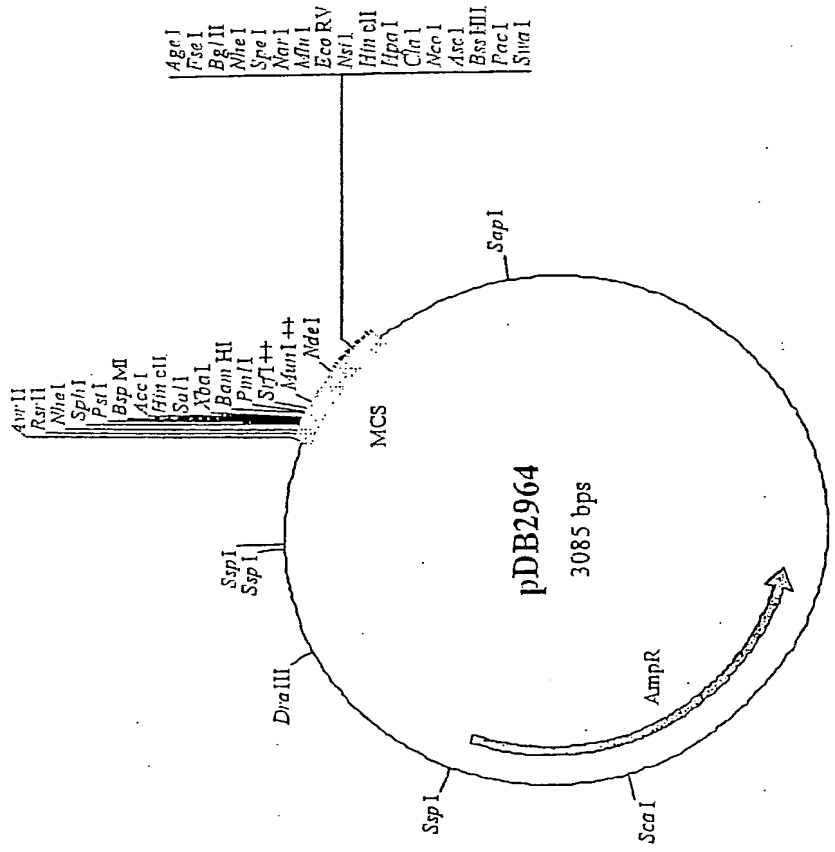


FIGURE 87

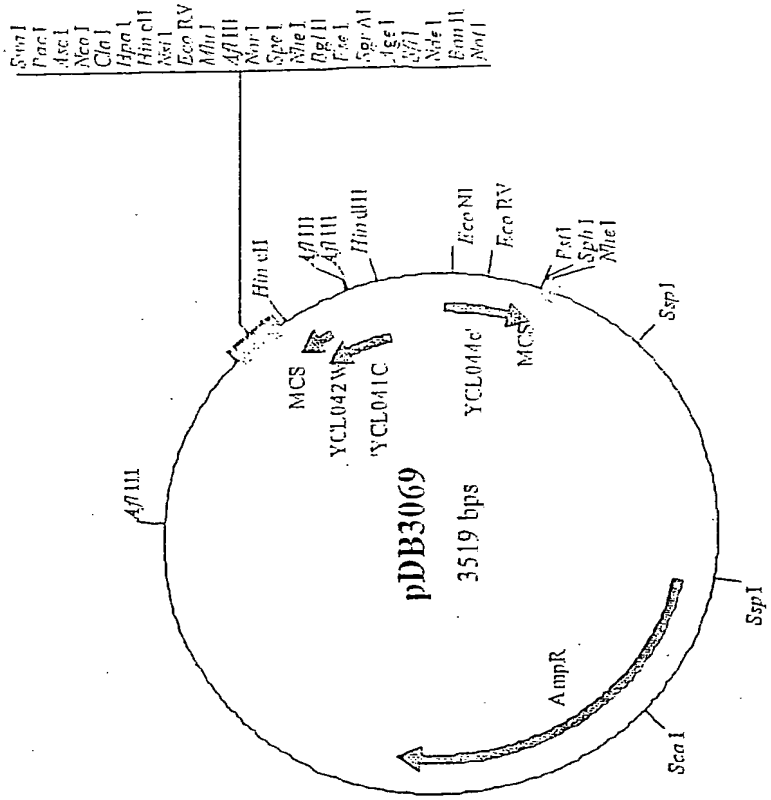


FIGURE 89

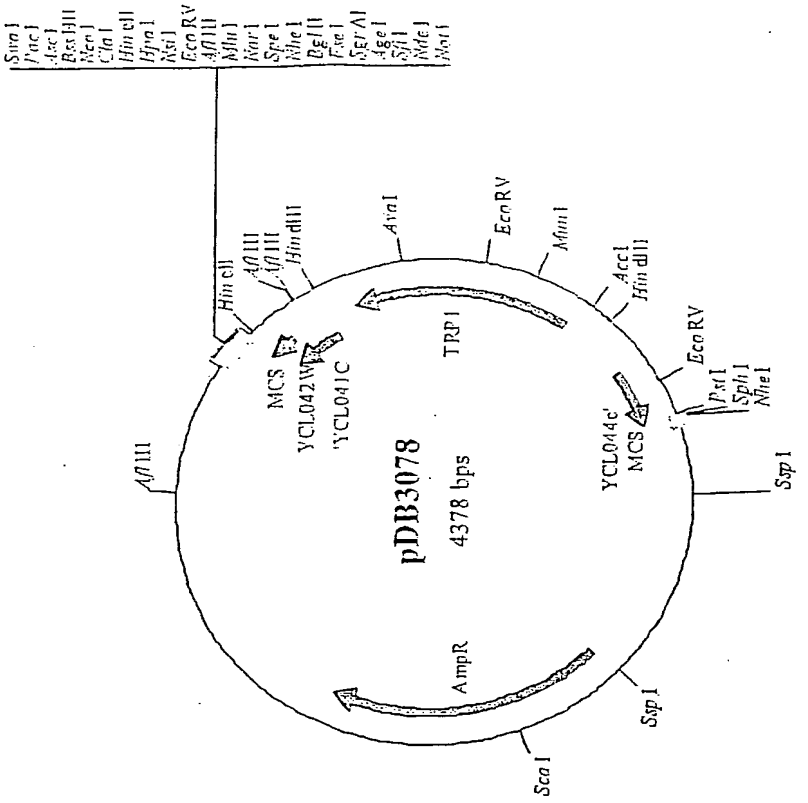


FIGURE 88

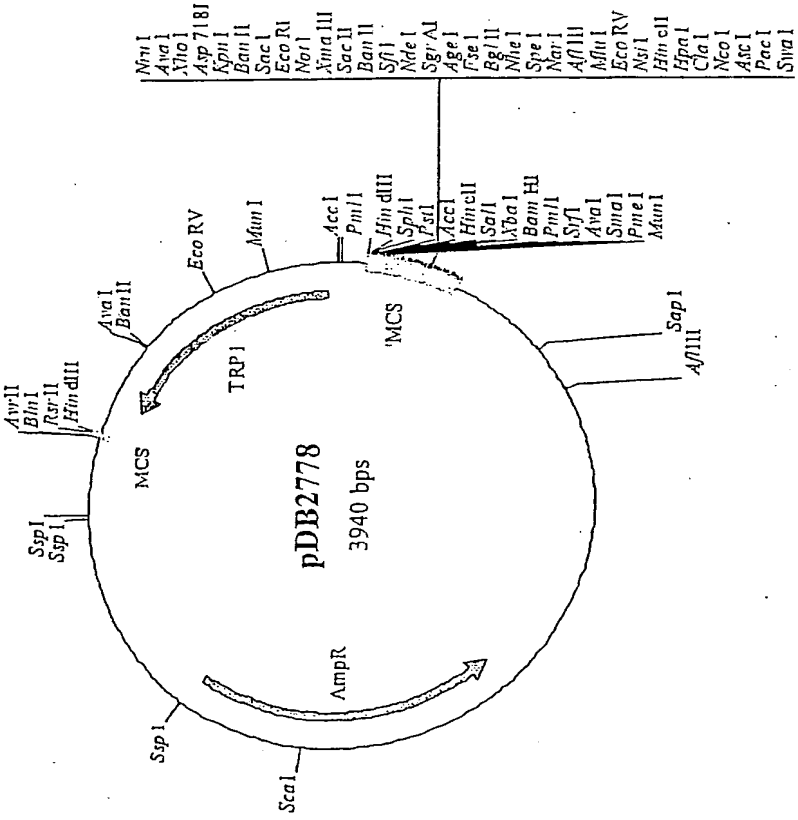


FIGURE 91

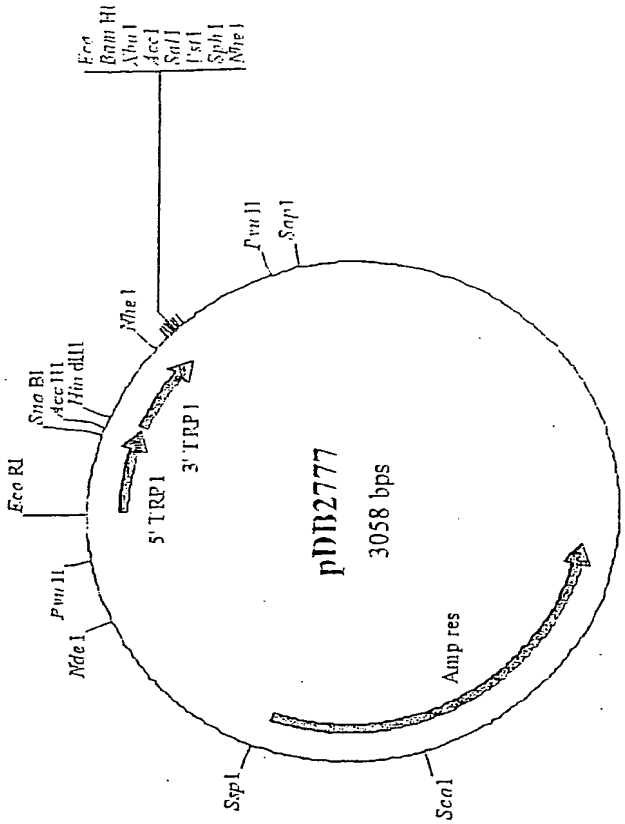


FIGURE 90

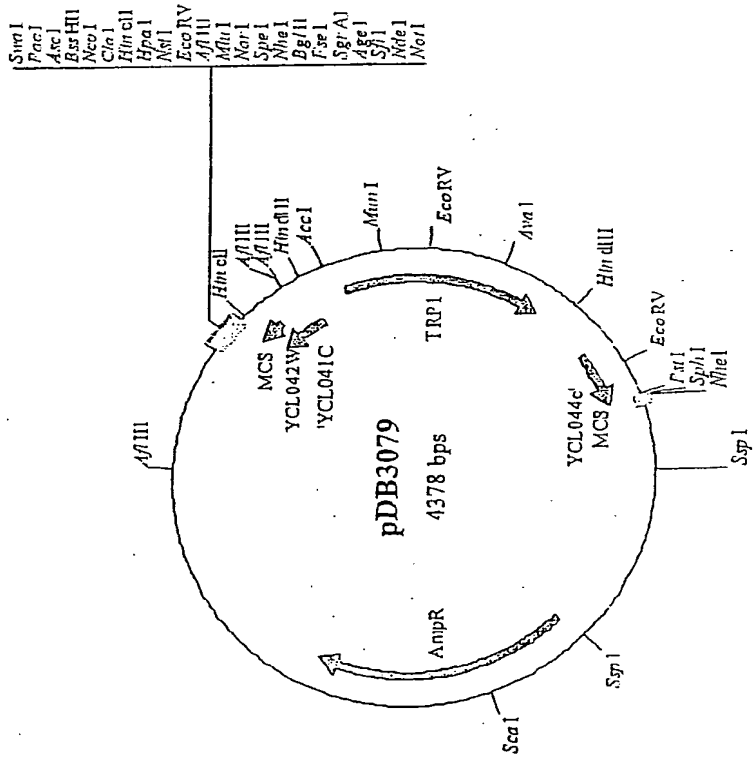


FIGURE 92

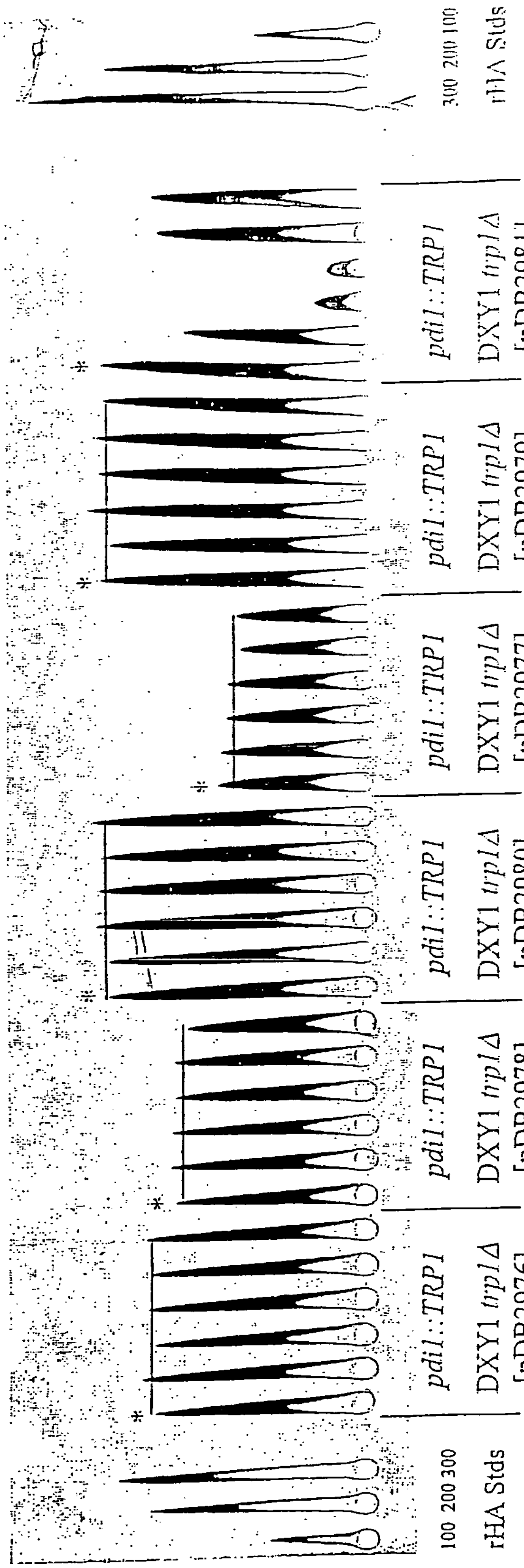

100 200 300
rHA Stds
pdi1::TRP1
DXY1 trp1Δ
[pDB2976]
pdi1::TRP1
DXY1 trp1Δ
[pDB2978]
pdi1::TRP1
DXY1 trp1Δ
[pDB2980]
pdi1::TRP1
DXY1 trp1Δ
[pDB2977]
pdi1::TRP1
DXY1 trp1Δ
[pDB2979]
pdi1::TRP1
DXY1 trp1Δ
[pDB2981]
300 200 100
rHA Stds

FIGURE 93

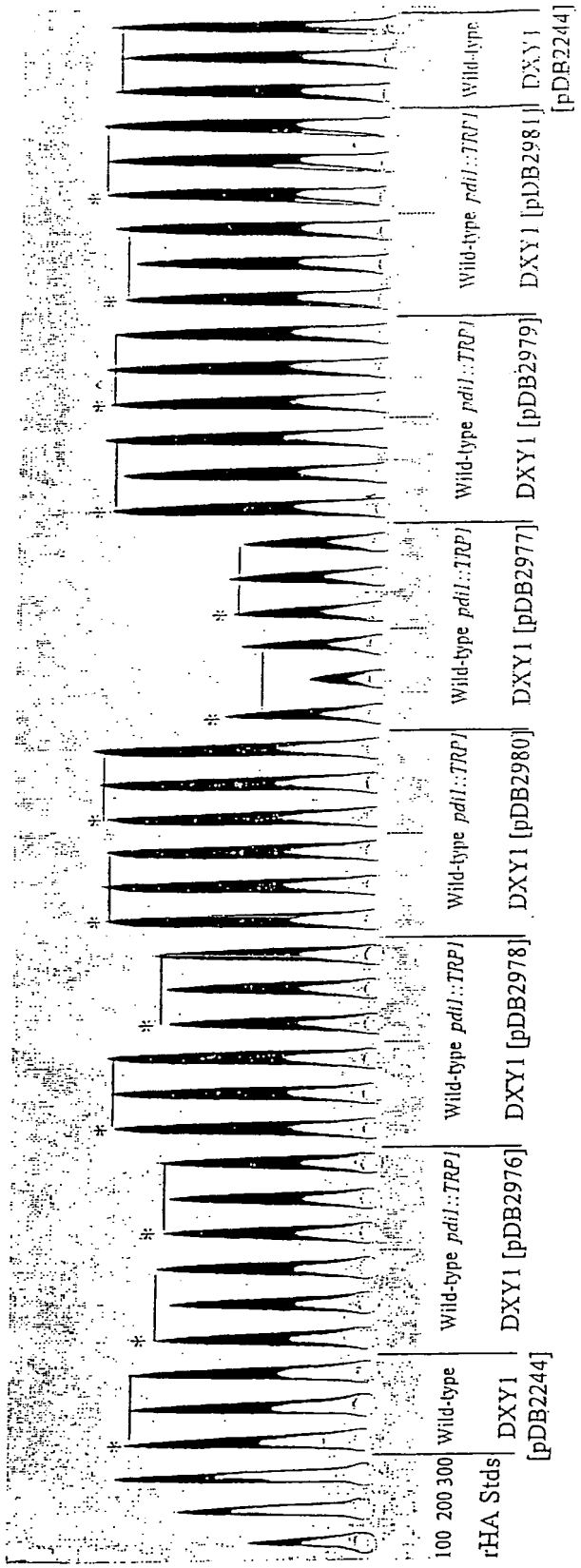

100 200 300
rHA Stds
Wild-type
DXY1 [pDB2244]
Wild-type pdi1::TRP1
DXY1 [pDB2976]
Wild-type pdi1::TRP1
DXY1 [pDB2978]
Wild-type pdi1::TRP1
DXY1 [pDB2980]
Wild-type pdi1::TRP1
DXY1 [pDB2977]
Wild-type pdi1::TRP1
DXY1 [pDB2979]
Wild-type pdi1::TRP1
DXY1 [pDB2981]
Wild-type
DXY1 [pDB2244]

Figure 94A

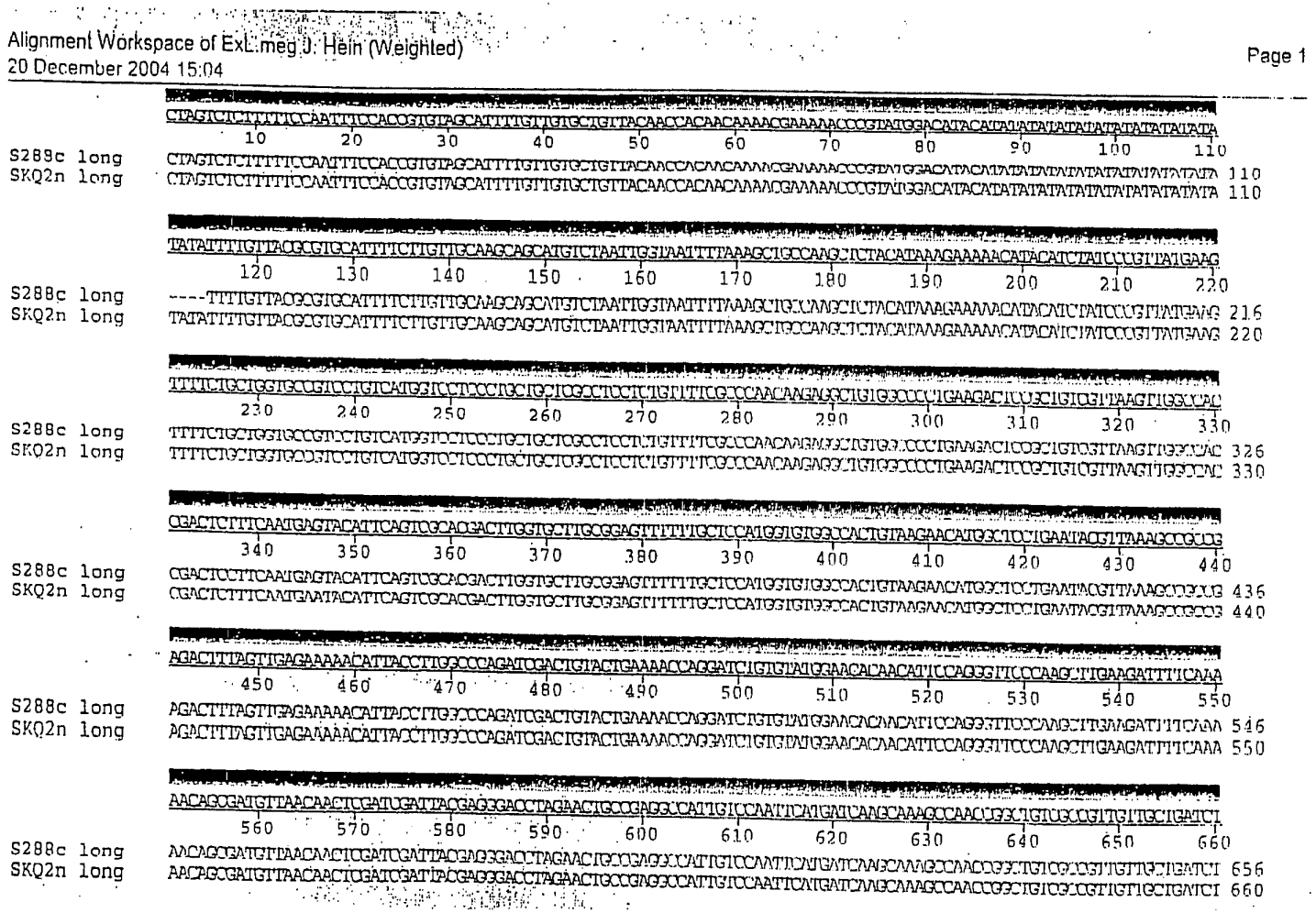
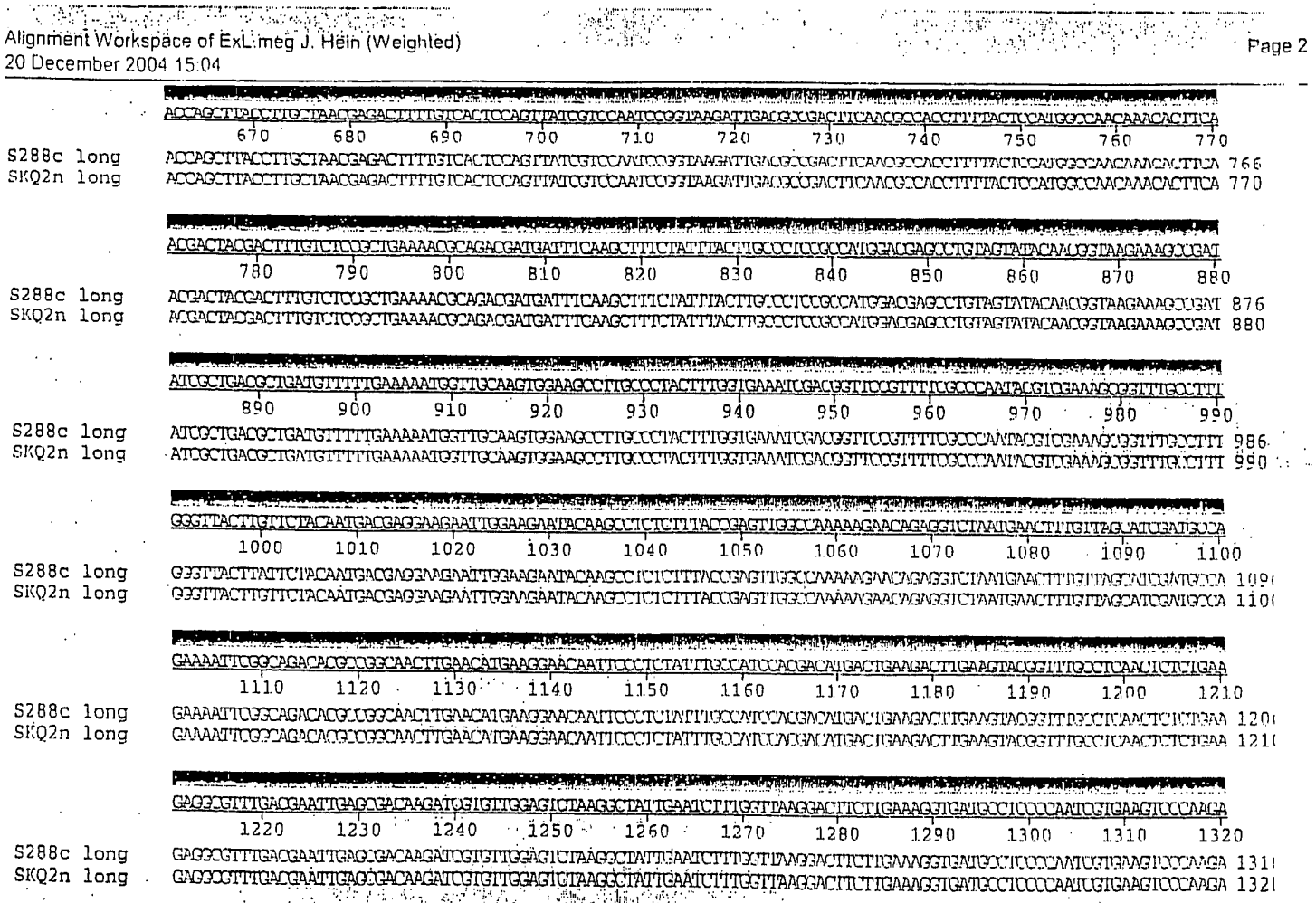

Alignment Workspace of ExL.meg J. Hein (Weighted)
20 December 2004 15:04
Page 1
S289c long
SKQ2n long
S288c long
SKQ2n long
S288c long
SKQ2n long
S288c long
SKQ2n long
S288c long
SKQ2n long

Figure 94B



WO 2005/061718
PCT/CA2004/002162

Figure 94C

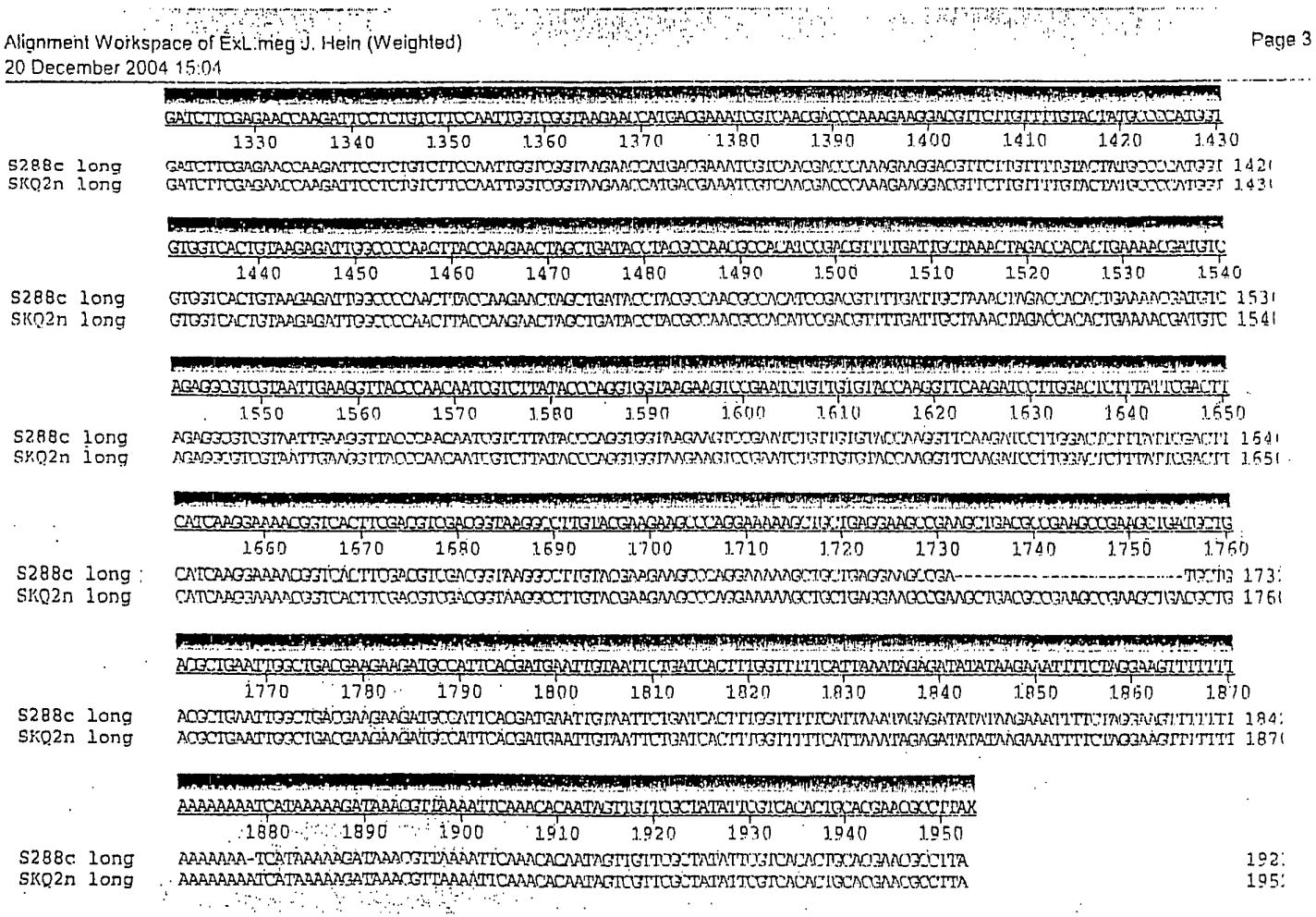

Alignment Workspace of ExL.meg J. Hein (Weighted)
20 December 2004 15:04
Page 3
S288c long
SKQ2n long

Figure 96

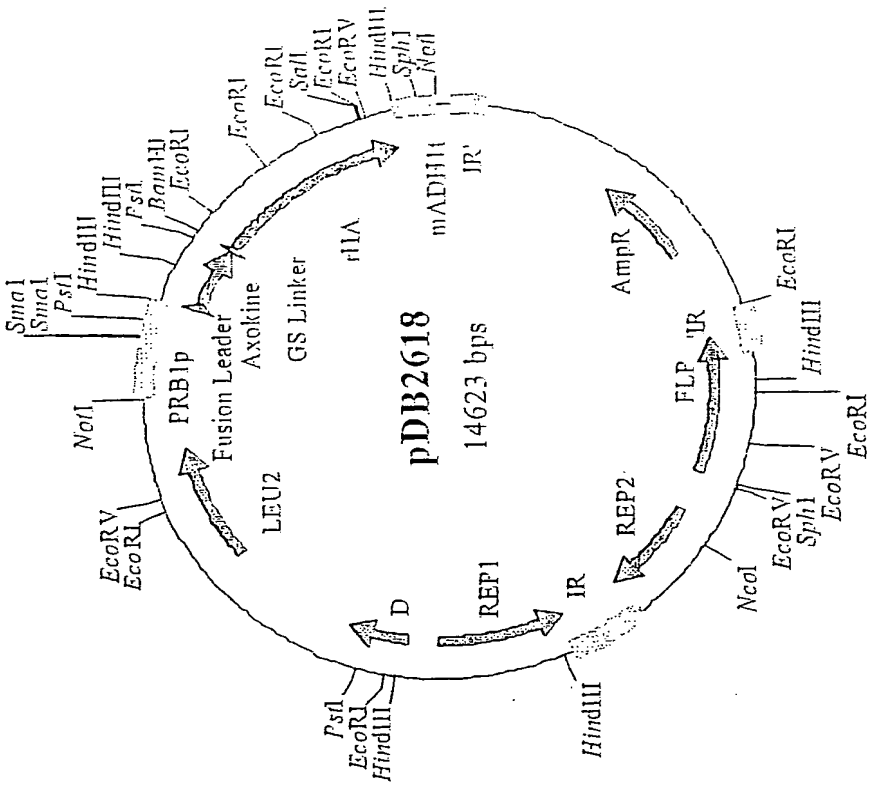


Figure 95